BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

Case 636-EPC

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 458 971 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
03.01.1996 Bulletin 1996/01

(21) Application number: **90917691.9**

(22) Date of filing: **04.12.1990**

(51) Int Cl.6: **C12N 9/12**, C12N 15/54, C12N 1/21, C12P 19/32 // (C12N9/12, C12R1:19), (C12N15/54, C12R1:19), (C12N1/21, C12R1:19), (C12P19/32, C12R1:19)

(86) International application number:
PCT/JP90/01567

(87) International publication number:
WO 91/08286 (13.06.1991 Gazette 1991/13)

(54) INOSINE GUANOSINE KINASE

INOSIN-GUANOSIN-KINASE

INOSINE GUANOSINE KINASE

(84) Designated Contracting States:
AT BE CH DE ES FR GB IT LI LU NL SE

(30) Priority: **05.12.1989 JP 315537/89**

(43) Date of publication of application:
04.12.1991 Bulletin 1991/49

(83) **Declaration under Rule 28(4) EPC (expert solution)**

(73) Proprietor: **KYOWA HAKKO KOGYO CO., LTD.**
Chiyoda-ku, Tokyo 100 (JP)

(72) Inventors:
- **MORI, Hideo**
  Tokyo 194 (JP)
- **IIDA, Akihiro**
  Tokyo 194 (JP)
- **TESHIBA, Sadao**
  Tokyo 194-01 (JP)
- **FUJIO, Tatsuro**
  Yamaguchi 747 (JP)

(74) Representative: **Casalonga, Axel et al**
D-80469 München (DE)

(56) References cited:
JP-A-57 110 194 **US-A- 4 152 209**

- **MOLECULAR AND GENERAL GENETICS vol. 143, no. 1, 1975, Berlin, DE, pages 85 - 91, BJARNE JOCHIMSEN et al.: 'Location on the chromosome of Escherichia coli of genes governing purine metabolism'**
- **JOURNAL OF GENERAL MICROBIOLOGY, Vol. 135, No. 5, (1989), B. HOVE-HENSEN et al.: 'Role of Guanosine Kinase in the Utilization of Guanosine for Nucleotide Synthesis in Escherichia coli', p. 1263-1271**
- **CHEMICAL ABSTRACTS, Vol. 68, (1968), K.J. PIERRE, et al.: 'Formation of inosine 5'-monophosphate by a kinase in cell-free extracts of Ehrlich ascites cell invitro', Abstract No. 102069a**

Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

EP 0 458 971 B1

Printed by Jouve, 75001 PARIS (FR)

Description

Technical Field

The present invention relates to an inosine-guanosine kinase which catalyzes the reaction of forming 5'-inosinic acid (5'-IMP) from inosine and adenosine triphosphate (ATP) or deoxyadenosine triphosphate (dATP) and the reaction of forming 5'-guanylic acid (5'-GMP) from guanosine and ATP or dATP, and to a process for producing 5'-nucleotides using the enzyme.

Background Art

5'-Nucleotides, inter alia, 5'-IMP and 5'-GMP have a flavor enhancing activity and are widely used as a seasoning agent. As the method for producing 5'-nucleotides, there are known a method for enzymatically decomposing RNA present in yeast cells (Journal of Agricultural and Chemical Society of Japan, 34, 489, 1969), a method for culturing a microorganism having an ability to produce 5'-IMP [Agricultural and Biological Chemistry, 46, 2557 (1982)], a method for chemical phosphorylation of nucleosides such as inosine and guanosine [Bulletin of the Chemical Society of Japan, 42, 3505-3508 (1969)], and the like. Currently, it is considered that the chemical phosphorylation utilizing these nucleosides would be the most advantageous from an industrial standpoint, because inosine and guanosine are easily obtained by fermentation. However, in the case of performing chemical phosphorylation, large quantities of chlorides are used at a lower temperature [Bulletin of the Chemical Society of Japan, 42, 3505-3508 (1969)] so that the method is not desired from viewpoints of both costs and environmental hygiene. Accordingly, enzymatic phosphorylation of nucleosides under mild conditions is desired. Hitherto, biochemical phosphorylation of purine nucleosides using cultured cells of various microorganisms has been reported (Japanese Published Unexamined Patent Application Nos. 116698/83 and 230094/88) but is inferior to chemical phosphorylation in terms of conversion rate and yield.

As an inosine-phosphorylating enzyme, inosine kinase (EC 2.7.1.73) is known but the crude active fraction derived from animal tissues or microorganisms is only reported [The Enzymes, Vol. IX, 54-56 (1973), Academic Press; Nucleosides and Nucleobases in Microorganisms, pp. 66, (1983), Academic Press]. The presence of a guanosine kinase activity which phosphorylates guanosine in E. coli is also suggested [J. Gen. Microbiol., 135, 1263-1273 (1989)]. However, the activity is low and it is extremely difficult to purify the enzyme so that it is far beyond industrial utilization. In addition, physicochemical properties of the enzyme are not clarified. In any event, inosine-guanosine kinase is an enzyme that has been neither isolated nor purified.

An object of the present invention is to provide an inosine-guanosine kinase and also to provide a method for preparing 5'-nucleotides, inter alia, 5'-IMP and 5'-GMP, which have been widely utilized as a seasoning agent, industrially at low costs.

Disclosure of the Invention

According to the present invention, there are provided an inosine-guanosine kinase derived from a microorganism belonging to the genus Escherichia which catalyzes the reaction of forming 5'-IMP from inosine and ATP or dATP and the reaction of forming 5'-GMP from guanosine and ATP or dATP, a process for producing the enzyme and a process for preparing 5'-nucleotides using the enzyme.

Hereafter the present invention is described in detail.

The inosine-guanosine kinase in accordance with the present invention can be obtained by culturing in a medium a microorganism belonging to the genus Escherichia having an ability to produce the inosine-guanosine kinase, producing and accumulating the inosine-guanosine kinase in cultured cells and recovering the inosine-guanosine kinase therefrom.

As the microorganism to be used, it may be any of the microorganisms which are obtainable from the natural world or constructed by genetic engineering so long as it belongs to the genus Escherichia and has an ability to produce the inosine-guanosine kinase.

Hereafter a method for obtaining the microorganism of the present invention constructed by genetic engineering and a method for producing the inosine-guanosine kinase using the microorganism are given.

The inosine-guanosine kinase may be obtained by isolating chromosomal DNA from a microorganism belonging to the genus Escherichia, cloning a gene encoding the inosine-guanosine kinase to produce a bacterial strain having an enhanced inosine-guanosine kinase activity using genetic engineering and culturing the strain.

Isolation and cloning of the gene encoding the inosine-guanosine kinase derived from a strain belonging to the genus Escherichia can be carried out as described below. That is, chromosomal DNA from strains of the genus Escherichia is isolated by a conventional method for isolation of DNA, for example, the phenol method [Biochim. Biophys. Acta, 72, 619-629 (1963)]. The resulting chromosomal DNA is cleaved with a suitable restriction enzyme, for example,

BamHI, Sau3AI, BglII, etc. By inserting the fragments cleaved with the restriction enzyme into a vector DNA, a recombinant DNA in which the DNA fragment containing the gene encoding the inosine-guanosine kinase derived from a strain of the genus Escherichia has been inserted can be obtained, together in a mixture with various other recombinant DNAs. In the recombinant DNA mixture described above is the recombinant DNA comprising the DNA fragment containing the gene encoding the inosine-guanosine kinase. Using the recombinant DNA mixture, a host microorganism is transformed according to the method of Cohen et al. [Proc. Natl. Acad. Sci. U.S.A., 69, 2110 (1979)].

As a source for the gene encoding the inosine-guanosine kinase, there is mentioned chromosomal DNA of a microorganism belonging to the genus Escherichia. Specific examples are Escherichia coli HM70 strain, Escherichia coli W3110 strain [Molecular Cloning, Cold Spring Harbor Laboratory (1982); ATCC 14948], etc.

As the vector harboring the DNA fragment containing the gene encoding the inosine-guanosine kinase inserted therein, any vector among phage vectors, plasmid vectors, etc. may be used as long as the vector is autonomously replicated in a strain belonging to the genus Escherichia. Preferred examples are pBR322 [Gene, 2, 95 (1977)], pUC19 [Gene, 33, 103 (1985)], pTrS30 [Doctoral Thesis by Tatsuya Nishi (1988), pp. 130, Tokyo University], etc.

Any host microorganism may be used as long as it belongs to the genus Escherichia and is capable of carrying a recombinant DNA obtained by inserting, into a vector, the gene encoding the inosine-guanosine kinase derived from a strain belonging to the genus Escherichia. Specific examples are Escherichia coli HM70 strain, Escherichia coli MC1000 strain [J. of Molecular Biology, 138, 179-207 (1980)], Escherichia coli DHI strain [Molecular Cloning, 505, Cold Spring Harbor Laboratory (1982)], etc.

The gene may also be inserted into another bacterial species by subcloning the gene from the thus obtained recombinant DNA containing the gene encoding the inosine-guanosine kinase derived from a strain belonging to the genus Escherichia, by use of the host-vector system of the other species.

As the host-vector system, all known systems may be used. Examples include the host-vector systems of the genus Serratia, the genus Corynebacterium, the genus Brevibacterium, the genus Pseudomonas, the genus Bacillus, etc.

Escherichia coli HM70 strain which is one of the sources for the gene encoding the inosine-guanosine kinase is a strain obtained by disrupting, on a genetic level, a part of the nucleoside-degrading activity of Escherichia coli SΦ609 strain [Molec. Gen. Genet., 143, 85-91 (1975)] obtained from National Genetics Research Institute, Japan, through treatment with UV rays. The HM70 strain is used not only as a source for the gene but also as a host for cloning of the gene.

Selection of HM70 strain was made as follows. SΦ609 strain has an inosine-degrading activity and therefore, when the strain is allowed to grow on a plate medium supplemented with inosine, it degrades inosine into hypoxanthine and ribose. Furthermore, the SΦ609 strain can use ribose as a sugar source and thus, when it is allowed to grow on a sugar metabolism assay plate supplemented with inosine (MacCONKEY plate supplemented with inosine), it assimilates ribose so that red colonies are formed. Therefore, SΦ609 strain is smeared on inosine-supplemented MacCONKEY plate and UV rays are irradiated to such a degree that a killing rate becomes about 95%, whereby mutation is induced. The appearing white colonies are strains having a lowered ribose productivity, namely, strains having a decreased inosine-degrading activity. A strain having the lowest inosine-degrading activity is selected from the colonies and named HM70 strain.

SΦ609 strain and HM70 strain cannot grow on the minimum agar plate medium supplemented with hypoxanthine since these strains lack the purine nucleotide biosynthesis pathway and the salvage pathway which produces 5'-IMP from hypoxanthine. However, HM70 strain having a decreased inosine-degrading activity which is about one fourth of that of SΦ609 strain can utilize inosine. Therefore, HM70 strain can grow even on inosine-supplemented minimum agar plate medium [plate medium prepared by dissolving 6 g of Na_2HPO_4, 3 g of KH_2PO_4, 0.5 g of NaCl, 1 g of NH_4Cl and 15 g of agar in 1 liter of distilled water, adjusting pH to 7.4 with 1N NaOH, then sterilized by autoclaving and then adding a sterile solution of 2 mℓ of 1M $MgSO_4$, 10 mℓ of 20% glucose and 0.1 mℓ of 1M $CaCl_2$ (inosine was supplemented in a final concentration of 5 mM)], while SΦ609 strain cannot grow on that medium. However, this growth restoration results from a weak inosine-guanosine kinase activity inherently possessed by HM70 strain and its growth rate is extremely low. When inosine-guanosine kinase structural gene ligated with a plasmid vector is introduced into this HM70 strain, the inosine-guanosine kinase activity in the cells increases and growth on the inosine-supplemented minimum agar plate medium is accelerated. On the other hand, since the parent strain SΦ609 has a strong inosine-degrading activity, even though a recombinant DNA comprising the inosine-guanosine kinase gene and a plasmid vector is introduced into the parent strain, the parent strain cannot grow on inosine-supplemented minimum agar plate medium because of degradation of inosine in the medium

From the thus obtained transformants, a strain having an activity which catalyzes the reaction of forming 5'-IMP from inosine and ATP or dATP and the reaction of forming 5'-GMP from guanosine and ATP or dATP is selected as follows.

The obtained transformants are treated with an organic solvent to afford membrane permeability thereto. Using the thus treated product as an enzyme source, the reaction of forming 5'-IMP from inosine and ATP or dATP or the reaction of forming 5'-GMP from guanosine and ATP or dATP is carried out. An amount of 5'-IMP or 5'-GMP produced is quan-

titatively determined by high performance liquid chromatography (HPLC). A strain which shows an increased amount of 5'-IMP or 5'-GMP produced is an inosine-guanosine kinase clone. Also when a transformant constructed by using HM70 strain as a host is inoculated on an inosine-supplemented minimum agar plate medium and kept at 30°C, a few colonies showing a fast growth appear in 2 to 3 days. Most of these strains showing a fast growth are inosine-guanosine kinase clones. From the obtained transformants, recombinant DNA molecules are isolated to obtain a recombinant DNA comprising the gene encoding the inosine-guanosine kinase derived from a strain belonging to the genus Escherichia.

Examples of the strains in which inosine-guanosine kinase activity has been enhanced include Escherichia coli HM70/pBM2 carrying a recombinant DNA constructed by inserting into a vector a gene encoding the inosine-guanosine kinase derived from Escherichia coli HM70 strain, Escherichia coli DHI/pBMI carrying a recombinant DNA constructed by inserting into a vector a gene encoding the inosine-guanosine kinase derived from Escherichia coli W3110, etc. Escherichia coli HM70/pBM2 and Escherichia coli DHI/pBMI have respectively been deposited as Escherichia coli HM72 and Escherichia coli HMI with the Fermentation Research Institute, Agency of Industrial Science & Technology, Japan, under the Budapest Treaty, as shown in Table 1.

Table 1

Indication of Microorganism Recognition	FERM BP-	Date of Deposit
Escherichia coli HM72	3125	October 6, 1990
Escherichia coli HM1	2669	December 1, 1989

These strains having an enhanced inosine-guanosine kinase activity are cultured in accordance with a conventional method for culturing bacteria. That is, the microorganism may be cultured in a conventional medium containing carbon sources, nitrogen sources, inorganic compounds, amino acids, vitamins, etc. under aerobic conditions, while controlling temperature, pH, etc.

As the carbon sources used in a medium, carbohydrates such as glucose, fructose, sucrose, molasses, blackstrap molasses, starch hydrolyzate, etc. alcohols such as ethanol, glycerine, sorbitol, etc., organic acids such as pyruvic acid, lactic acid, acetic acid, etc. amino acids such as glycine, alanine, glutamic acid, aspartic acid, etc. can be used so long as the microorganism can assimilate them. A concentration of these carbon sources used is preferably 5 to 30%.

As the nitrogen sources, ammonia, ammonium salts of various inorganic and organic compounds such as ammonium chloride, ammonium sulfate, ammonium nitrate, ammonium carbonate, ammonium acetate, ammonium phosphate, etc. nitrogen-containing organic compounds such as urea, peptone, NZ amine, meat extract, yeast extract, corn steep liquor, casein hydrolyzate, fish meal or its digested products, etc. various amino acids such as glycine, glutamic acid, etc. can be used. A concentration of these nitrogen sources used is generally 0.1 to 10%.

As the inorganic compounds, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, magnesium sulfate, magnesium phosphate, sodium chloride, ferrous sulfate, manganese sulfate, zinc sulfate, calcium carbonate, etc. can be used.

When the microorganism to be used requires a specific nutrient such as amino acid, nucleic acid, vitamin, etc. for its growth, these substances are supplemented to the medium in a suitable amount.

Culturing is carried out under aerobic conditions such as shaking culture, agitation submerged culture, etc. A preferred temperature for culturing is generally 28 to 32°C. A period for culturing is generally 1 to 24 hours. A pH of medium is desirably kept neutral with ammonia, urea, a sodium hydroxide solution, etc.

After the completion of the culturing, the inosine-guanosine kinase may be isolated from the cultured cells in a conventional manner for collecting an enzyme. for example. as follows. Firstly the resulting cells are thoroughly washed and then ultrasonicated to give cell-free extract. After centrifugation. protamine sulfate is added to the resulting supernatant. The mixture is centrifuged to remove high molecular nucleic acid as precipitates. The supernatant is added to Sephadex G-50 followed by desalting through gel filtration. Subsequently. an anion exchange chromatography treatment using DEAE Sepharose and gel filtration through Sephacryl S-200 are carried out to give the purified product.

Components of each buffer in the specification refer to the following compounds

HEPES: N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid
PIPES: piperazine-N.N'-bis(2-ethanesulfonic acid)
TAPS : N-tris(hydroxymethyl)methyl-3-amino-1-propanesulfonic acid
CHES: cyclohexylaminoethanesulfonic acid

The activity of the inosine-guanosine kinase obtained is determined as follows. A reaction solution (hereafter referred to as reaction solution a) having a composition of 100 mM HEPES buffer (pH 7.2). 10 mM $MgSO_4$, 50 mM KCl. 1 mM ATP and 1 mM inosine is brought into contact with the inosine-guanosine kinase in a concentration of about 10 μg protein/mℓ followed by reaction at 30°C for about 30 minutes. During the course of reaction, a part of the reaction solution

is subjected to sampling intermittently. After the reaction solution is diluted to 1/20 with 0.2M NaH_2PO_4 (adjusted the pH to 2.6 with H_3PO_4) to stop the reaction, an amount of 5'-IMP in the stopped reaction solution is quantitatively determined by HPLC. Analysis by HPLC is made by using 0.2M NaH_2PO_4 (pH 2.6) at a flow rate of 1 mℓ/min. as an eluting solution and Asahipak GS-320H (manufactured by Asahi Chemical Co., Ltd.) as a column. Detection of components was made by using the UV absorbance at 254 nm as an index. Quantitative determination was made by comparing absorbancy with that of the standard.

Next, physicochemical properties of the obtained inosine-guanosine kinase are described below.

(1) Action

The enzyme has an action of forming ADP and 5'-IMP from ATP and inosine, an action of forming dADP and 5'-IMP from dATP and inosine, an action of forming ADP and 5'-GMP from ATP and guanosine, and an action of forming dADP and 5'-GMP from dATP and guanosine.

(2) Optimum pH

A reaction was carried out at 30°C for 20 minutes in a manner similar to the method for determination of inosine-guanosine kinase activity described above, except that PIPES (pH 6.6-7.1), HEPES (pH 6.9-8.3), TAPS (pH 7.9-8.8) and CHES (pH 8.7-10.1) were used in replacement of the buffer component (100 mM) in the composition of reaction solution a. As the result, the optimum pH was 6.9-8.2.

(3) pH Stability

This enzyme is treated at 4°C for 16 hours in an aqueous solution containing 50 mM buffer (CHES; pH 10.0-9.0, TAPS; pH 8.2, HEPES; pH 8.3-7.3 or PIPES; pH 6.6) and 5 mM β-mercaptoethanol under both conditions in the absence of KCl and in the presence of 250 mM KCl. After the treatment, the activity is determined. When treated in a pH range of 6.6 to 9.0, the treated enzyme maintains the residual activity of 90% or more of the activity of intact standard enzyme and stably keeps its activity in a pH range of 6.6 to 9.0. The addition of KCl ameliorates storage stability.

(4) Optimum temperature

In the method for determination of inosine-guanosine kinase activity described above, the activity is determined by varying the temperature of 0 to 50°C and 31 to 41°C. The optimum temperature is 25 to 40°C.

(5) Temperature stability

A reaction solution composed of 20% glycerol, 50 mM Tris-hydrochloride buffer (pH 8), 5 mM β-mercaptoethanol and 100 mM NaCl is contacted with inosine-guanosine kinase in a concentration of about 10 μg protein/mℓ and treated at a temperature of 26 to 50°C for 15 minutes to determine the residual activity at each temperature. As the result, since treatment at 40°C or lower inactivates the enzyme by only 20% or less, the enzyme is stable up to 40°C. In contrast, the activity is rapidly lost by treatment at 50°C.

(6) Substrate specificity

In the method for determination of inosine-guanosine kinase activity described above, a reaction is carried out at 30°C by changing the concentration of KCl to 300 mM in the composition of reaction solution a and adding various neutralized phosphate sources to the reaction solution instead of ATP in a final concentration of 5 mM. As shown in Fig. 1, the reaction proceeds well when using ATP or dATP as substrate for phosphate-donor. Further in the case of uridine triphosphate (UTP), the reaction proceeds a little bit. However, adenosine diphosphate (ADP), adenosine monophosphate (5'-AMP), guanosine triphosphate (GTP), orotic acid monophosphate (5'-OMP), cytidine monophosphate (5'-CMP), p-nitrophenyl phosphate (PNPP), acetylphosphoric acid, tripolyphosphoric acid, tetrapolyphosphoric acid, pyrophosphoric acid and phosphoric acid could not act as phosphate-donor substrates for this enzyme in this reaction. This indicates that the enzyme is classified in an enzyme group called kinases and clearly distinguished from enzymes which govern transphosphorylation used in Japanese Published Unexamined Patent Application Nos. 119898/83 and 230094/88, etc., namely, nucleoside phosphotransferases (EC 2.7.1.77). Where ATP or dATP is used as phosphate-donor, inosine and guanosine are preferred as phosphate-acceptor.

(7) Inhibitors

In the method for determination of inosine-guanosine kinase activity described above, the activity is determined by reacting at 30°C for 30 minutes except that the concentration of KCl is changed to 300 mM and 1 mM metal salt is added to the composition of reaction solution a. The activity is determined as a standard when no metal salt is added. The results are shown in Table 2. The enzyme is inhibited by metal ions such as Co^{2+}, Cu^{2+}, Zn^{2+}, etc.

Table 2

Metal Salt (1 mM)	Relative Activity (%)
None	100
$FeCl_2$	112
$FeCl_3$	131
$CaCl_2$	107
$CoCl_2$	32
$CuCl_2$	1
$MnCl_2$	134
$BaCl_2$	116
$ZnSO_4$	5
$Zn(CH_3COO)_2$	5
NaCl	104
NaF	96

(8) Activation

In the method for determination of inosine-guanosine kinase activity described above, a reaction is carried out by changing the buffer to Tris-hydrochloride buffer (pH 8.0), and varying the concentration of KCl in the composition of reaction solution a. The results are shown in Fig. 2. When 100 mM NaCl is added instead of KCl, no reaction proceeds. This reveals that the enzyme requires K^+ for activation. Next, activation is examined in the system where the concentration of KCl is changed to 300 mM in the composition of reaction solution a, by adding various divalent or trivalent metal ion salts instead of $MgSO_4$. As shown in Table 3, Mg^{2+} and Mn^{2+} exhibit an activating action. It is thus revealed that this enzyme requires one of two combinations: one is K^+ and Mg^{2+} and the other is K^+ and Mn^{2+}.

Table 3

Metal Salt (10 mM)	Produced IMP (mM)
None	0
$FeCl_2$	0
$FeCl_3$	0
$CaCl_2$	0
$CoCl_2$	0.01
$CuCl_2$	0
$MnCl_2$	0.51
$BaCl_2$	0
$ZnSO_4$	0
$Zn(CH_3COO)_2$	0
$MgCl_2$	0.60
$MnSO_4$	0.64
$MgSO_4$	0.68

(9) Km value

Km value in the reaction solution composed of 2 mM ATP, 10 mM $MgSO_4$, 300 mM KCl and 0.1 M HEPES buffer (pH 7.2) was 2.1 mM for inosine and 6.1 μM for guanosine.

(10) Amino acid sequence and nucleotide sequence

Nucleotide sequence of the structural gene coding for this enzyme was determined by the dideoxy chain terminator method [Science, 214, 1205-1210 (1981), Gene, 19, 269-276 (1982)] and its amino acid sequence was deduced from the nucleotide sequence. The amino acid sequence and the nucleotide sequence are shown in Tables 4 and 5, respectively.

Table 4

```
  1  MetLysPheProGlyLysArgLysSerLysHisTyrPheProValAsnAlaArgAspPro
 21  LeuLeuGlnGlnPheGlnProGluAsnGluThrSerAlaAlaTrpValValGlyIleAsp
 41  GlnThrLeuValAspIleGluAlaLysValAspAspGluPheIleGluArgTyrGlyLeu
 61  SerAlaGlyHisSerLeuValIleGluAspAspValAlaGluAlaLeuTyrGlnGluLeu
 81  LysGlnLysAsnLeuIleThrHisGlnPheAlaGlyGlyThrIleGlyAsnThrMetHis
101  AsnTyrSerValLeuAlaAspAspArgSerValLeuLeuGlyValMetCysSerAsnIle
121  GluIleGlySerTyrAlaTyrArgTyrLeuCysAsnThrSerSerArgThrAspLeuAsn
141  TyrLeuGlnGlyValAspGlyProIleGlyArgCysPheThrLeuIleGlyGluSerGly
161  GluArgThrPheAlaIleSerProGlyHisMetAsnGlnLeuArgAlaGluSerIlePro
181  GluAspValIleAlaGlyAlaSerAlaLeuValLeuThrSerTyrLeuValArgCysLys
201  ProGlyGluProMetProGluAlaThrMetLysAlaIleGluTyrAlaLysLysTyrAsn
221  ValProValValLeuThrLeuGlyThrLysPheValIleAlaGluAsnProGlnTrpTrp
241  GlnGlnPheLeuLysAspHisValSerIleLeuAlaMetAsnGluAspGluAlaGluAla
261  LeuThrGlyGluSerAspProLeuLeuAlaSerAspLysAlaLeuAspTrpValAspLeu
281  ValLeuCysThrAlaGlyProIleGlyLeuTyrMetAlaGlyPheThrGluAspGluAla
301  LysArgLysThrGlnHisProLeuLeuProGlyAlaIleAlaGluPheAsnGlnTyrGlu
321  PheSerArgAlaMetArgHisLysAspCysGlnAsnProLeuArgValTyrSerHisIle
341  AlaProTyrMetGlyGlyProGluLysIleMetAsnThrAsnGlyAlaGlyAspGlyAla
361  LeuAlaAlaLeuLeuHisAspIleThrAlaAsnSerTyrHisArgSerAsnValProAsn
381  SerSerLysHisLysPheThrTrpLeuThrTyrSerSerLeuAlaGlnValCysLysTyr
401  AlaAsnArgValSerTyrGlnValLeuAsnGlnHisSerProArgLeuThrArgGlyLeu
421  ProGluArgGluAspSerLeuGluGluSerTyrTrpAspArg
```

Table 5

```
   1  ATGAAATTTC CCGGTAAACG TAAATCCAAA CATTACTTCC CCGTAAACGC
  51  ACGCGATCCG CTGCTTCAGC AATTCCAGCC AGAAAACGAA ACCAGCGCTG
 101  CCTGGGTAGT GGGTATCGAT CAAACGCTGG TCGATATTGA AGCGAAAGTG
 151  GATGATGAAT TTATTGAGCG TTATGGATTA AGCGCCGGGC ATTCACTGGT
 201  GATTGAGGAT GATGTAGCCG AAGCGCTTTA TCAGGAACTA AAACAGAAAA
 251  ACCTGATTAC CCATCAGTTT GCGGGTGGCA CCATTGGTAA CACCATGCAC
 301  AACTACTCGG TGCTCGCGGA CGACCGTTCG GTGCTGCTGG GCGTCATGTG
 351  CAGCAATATT GAAATTGGCA GTTATGCCTA TCGTTACCTG TGTAACACTT
 401  CCAGCCGTAC CGATCTTAAC TATCTACAAG GCGTGGATGG CCCGATTGGT
 451  CGTTGCTTTA CGCTGATTGG CGAGTCCGGG GAACGTACCT TTGCTATCAG
 501  TCCAGGCCAC ATGAACCAGC TGCGGGCTGA AAGCATTCCG GAAGATGTGA
 551  TTGCCGGAGC CTCGGCACTG GTTCTCACCT CATATCTGGT GCGTTGCAAG
 601  CCGGGTGAAC CCATGCCGGA AGCAACCATG AAAGCCATTG AGTACGCGAA
 651  GAAATATAAC GTACCGGTGG TGCTGACGCT GGGCACCAAG TTTGTCATTG
 701  CCGAGAATCC GCAGTGGTGG CAGCAATTCC TCAAAGATCA CGTCTCTATC
 751  CTTGCGATGA ACGAAGATGA AGCCGAAGCG TTGACCGGAG AAAGCGATCC
 801  GTTGTTGGCA TCTGACAAGG CGCTGGACTG GGTAGATCTG GTGCTGTGCA
 851  CCGCCGGGCC AATCGGCTTG TATATGGCGG GCTTTACCGA AGACGAAGCG
 901  AAACGTAAAA CCCAGCATCC GCTGCTGCCG GGCGCTATAG CGGAATTCAA
 951  CCAGTATGAG TTTAGCCGCG CCATGCGCCA CAAGGATTGC CAGAATCCGC
1001  TGCGTGTATA TTCGCACATT GCGCCGTACA TGGGCGGGCC GGAAAAAATC
1051  ATGAACACTA ATGGAGCGGG GGATGGCGCA TTGGCAGCGT TGCTGCATGA
1101  CATTACCGCC AACAGCTACC ATCGTAGCAA CGTACCAAAC TCCAGCAAAC
1151  ATAAATTCAC CTGGTTAACT TATTCATCGT TAGCGCAGGT GTGTAAATAT
1201  GCTAACCGTG TGAGCTATCA GGTACTGAAC CAGCATTCAC CTCGTTTAAC
1251  GCGCGGCTTG CCGGAGCGTC AAGACAGCCT GGAAGAGTCT TACTGGGATC
1301  GT
```

10 amino acids sequence of the enzyme at the N-terminal were determined using Amino Acid sequencer of Applied

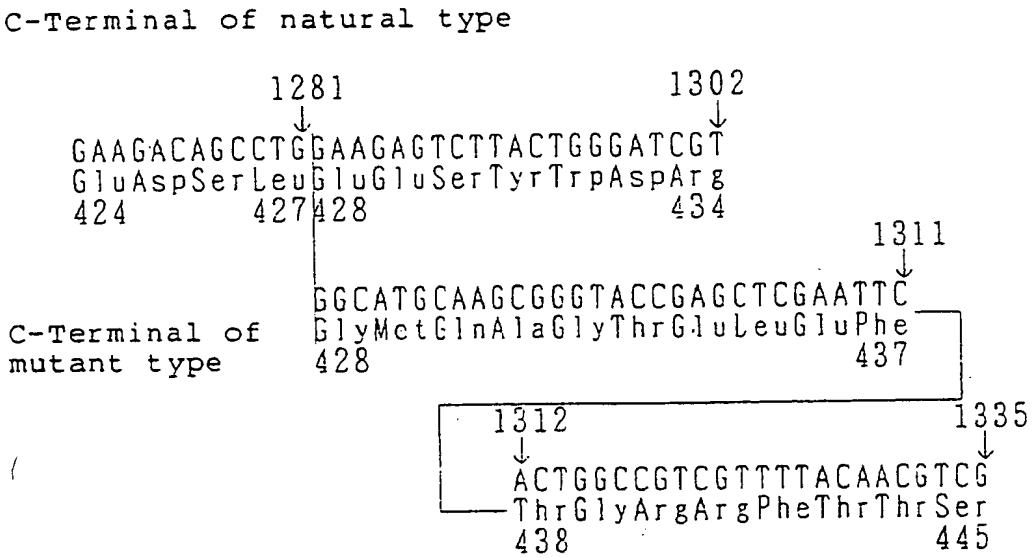
Biosystem Co., Ltd. The amino acid sequence coincided with the amino acid sequence (Table 4) deduced from the nucleotide sequence shown in Table 5. Methionine which is the first amino acid of this enzyme is not cut out. Furthermore, C-terminal peptide was isolated and collected from peptide groups obtained by digesting the enzyme with lysyl endopeptidase and amino acid sequence of the C-terminal peptide was determined by the method described above. The amino acid sequence coincided with the C-terminal amino acid sequence shown in Table 4. The foregoing reveals that the amino acid sequence of this enzyme coincides with the amino acid sequence (Table 4) deduced from the nucleotide sequence shown in Table 5.

(11) Molecular weight

Molecular weight deduced from the amino acid sequence is about 48400 daltons; according to measurement by SDS-polyacrylamide electrophoresis (manufactured by Biorad Co., Ltd., standard for low molecular weight, catalogue No. 161-0304 was used), molecular weight is about 43000 daltons.

The nucleotide sequence shown in Table 5 corresponds to the inosine-guanosine kinase represented by the amino acid sequence shown in Table 4. However, unless the inosine-guanosine kinase activity is lost, a strain carrying a recombinant DNA inserted with a gene obtained by modifying the nucleotide sequence may similarly be used as a strain having an increased inosine-guanosine kinase activity. Even a mutated enzyme produced by modifying guanine at the 1282nd position and its subsequent nucleotide sequence as shown in Table 6 exhibits an inosine-guanosine kinase activity.

Table 6



Next, mention may be made of process for producing 5'-nucleotides using the enzyme which catalyzes the reaction of forming 5'-nucleotides from nucleosides and ATP or dATP.

5'-nucleotides can be obtained by contacting nucleosides with ATP or dATP in an aqueous medium in the presence of an enzyme source having the enzyme activity which catalyzes the reaction of forming 5'-nucleotides from nucleosides and ATP or dATP.

As the nucleoside, inosine or guanosine is mentioned. As the 5'-nucleotides, 5'-IMP or 5'-GMP is mentioned.

As the enzyme source, inosine-guanosine kinase, bacterial cells having an ability to produce the enzyme, bacterial cells carrying a recombinant DNA obtained by inserting into a vector a DNA fragment containing a gene encoding the enzyme involved in the reaction of forming 5'-nucleotides from nucleosides and ATP or dATP, a culture thereof or treated matters thereof, etc. may be used.

As the treated matters, a concentrate of the culture or a dried culture, a culture treated with surfactant and/or organic solvent or lytic enzyme, bacterial cells obtained by centrifuging the culture, dried bacterial cells, acetone-treated cells, cells treated with surfactants and/or organic solvents, lytic enzyme-treated cells, immobilized bacterial cells or enzyme preparation extracted from bacterial cells, etc. may be used.

As the inosine and guanosine to be used in the reaction, any of purified or crude products, fermentation broth or cell-free supernatant of inosine and guanosine can be used for the reaction so long as they do not interfere the reaction

of forming 5'-nucleotides. The concentration of nucleosides is in the range of from 10 to 80 g/ℓ.

As the phosphate donor, there are ATP or dATP. Any of purified and crude ATP and dATP or materials containing AT or dATP may be used as ATP or dATP source so long as they do not contain any substance that interferes the reaction.

Since ATP and dATP are expensive, it is advantageous to add a microorganism having an ATP regenerating activity (Japanese Published Unexamination Patent Application No. 74595/86) to the reaction system, and to synthesize ATP from glucose and inorganic phosphoric acid.

In this case, an ATP precursor, an ATP regeneration energy donor, a phosphate donor and a microorganism having an ATP biosynthesis activity are allowed to exist in the reaction solution, instead of ATP. When the reaction system coupled with the ATP regeneration system is used, it is sufficient to use ATP in a catalytic amount (1.0 g/ℓ or less). When a necessary amount is supplied and brought into the reaction system from bacteria or a culture, it is unnecessary to particularly supplement ATP. Examples of the strains having an ATP regenerating activity are Brevibacterium ammoniagenes KY13761 strain [Agric. Biol. Chem., 42, 399-405 (1978)], Brevibacterium ammoniagenes ATCC 21477 strain, etc.

Brevibacterium ammoniagenes KY13761 produce inosine by fermentation, Therefore, when inosine fermentation broth (containing cells) obtained by culturing this KY13761 strain is used as a source of nucleoside and as a source of ATP regeneration, inosine in the fermentation broth can directly be phosphorylated without requiring purification of inosine, so that an efficient process for producing 5'-IMP can be provided in a less expensive way.

The reaction for forming 5'-nucleotides from inosine or guanosine, and ATP or dATP is carried out at a temperature of 20 to 40°C for 1 to 48 hours while adjusting pH to 6-8, preferably by adding a surfactant and/or an organic solvent. As the surfactant useful for treating the bacterial cells and for the reaction, there may be used surfactants including cationic surfactants such as polyoxyethylene stearylamine (e.g., Nymeen S-215, manufactured by Nippon Oil and Fats, Co., Ltd.; hereafter the same shall be used unless otherwise indicated), cetyltrimethylammonium bromide, cation FB, cation F2-40E, etc.; anionic surfactants such as sodium oleylamide sulfate, Newrex TAB, Rapizole 80, etc.; amphoteric surfactants such as polyoxyethylene sorbitan monostearate (e.g., Nonion ST221), etc.; tertiary amine PB, hexadecyldimethylamine, etc.; any other surfactants may be used so long as they accelerate the reaction of forming 5'-nucleotides from nucleosides and ATP or dATP. These surfactants may be used generally in a concentration of 0.1 to 50 mg/mℓ, preferably 1 to 20 mg/mℓ.

As the organic solvent, toluene, xylene, aliphatic alcohols, benzene, ethyl acetate may be used. The organic solvent may be used generally in a concentration of 0.1 to 50 μℓ/mℓ, preferably 1 to 20 μℓ/mℓ.

After completion of the reaction, 5'-nucleotides produced and accumulated in the reaction solution are obtained in a conventional manner using ion exchange resin, etc.

Brief Description of the Drawings

Fig. 1 relates to substrate specificity for inosine-guanosine kinase, wherein the ordinate represents an amount of 5'-TMP produced and the abscissa represents time.

Fig. 2 relates to KCl dependency of inosine-guanosine kinase, wherein the ordinate represents an amount of 5'-IMP produced and the abscissa represents an amount of KCl added.

Fig. 3 is a restriction enzyme map of plasmid pBM2 containing a gene encoding inosine-guanosine kinase derived from Escherichia coli HM70 strain.

Fig. 4 is a restriction enzyme map of mutant plasmid pBM Δ14 in which deletion in the structural gene of inosine-guanosine kinase has occurred up to the 5' end.

Fig. 5 is a restriction enzyme map of mutant plasmid pBMM5 in which deletion in the structural gene of inosine-guanosine kinase has occurred up to the 3' end.

Fig. 6 is a restriction enzyme map of vector pTrS30.

Fig. 7 is a restriction enzyme map of plasmid pIK75 which efficiently expresses inosine-guanosine kinase.

Fig. 8 shows a nucleotide sequence at the junction between the tryptophan promoter and the structural gene of inosine-guanosine kinase.

Fig 9 is a restriction enzyme map of plasmid pBMI containing a gene encoding inosine-guanosine kinase derived from Escherichia coli W3110 strain

Best Mode for Practising the Invention

In the following examples. reagents and vectors used in genetic engineering experiments are all manufactured by Takara Shuzo Co.. Ltd. Other reagents are those manufactured by Nakarai Tesque Co., Ltd.

Example 1

(1) Isolation of DNA

Escherichia coli HM70 strain was inoculated into LB liquid medium [1% trypton, 0.5% yeast extract and 1% sodium chloride (pH 7.5)] followed by culturing at 37°C overnight. After 15 g of the cultured cells was suspended in 120 mℓ of 20 mM Tris-hydrochloride buffer (pH 7.5) containing 2 mM EDTA, 15 mℓ of lysozyme solution (20 mg/mℓ lysozyme was dissolved in 20 mM Tris-hydrochloride buffer (pH 7.5) containing 2 mM EDTA) was added to the suspension. The mixture was allowed to stand at 30°C for an hour. Then 15 mℓ of 20% sodium laurylsulfate was added thereto and the mixture was slowly stirred. Next, 150 mℓ of phenol saturated with 10 mM Tris-hydrochloride buffer containing 1 mM EDTA was added to the solution followed by thoroughly stirring. The solution was centrifuged and 150 mℓ of the aqueous layer was fractionated. Such procedure for the phenol extraction was repeated 3 times. To 150 mℓ of the obtained aqueous layer were added 15 mℓ of 2.5 M sodium acetate solution and further 300 mℓ of ethanol. The precipitated chromosomal DNA was wound up around a glass rod and dried. Then, chromosomal DNA was dissolved in 30 mℓ of 10 mM Tris-hydrochloride buffer containing 1 mM EDTA and 50 μg/mℓ ribonuclease was added to the solution. The mixture was allowed to stand at 37°C for 30 minutes. After the same procedure for phenol extraction as described above was performed, 3 mℓ of 2.5 M sodium acetate solution and 60 mℓ of ethanol were added to the aqueous layer followed by standing at -20°C for 16 hours. After centrifugation, the obtained pellet was washed with 70% ethanol solution and dried to give purified chromosomal DNA. The chromosomal DNA was suspended in 10 mM Tris-hydrochloride buffer containing 1 mM EDTA.

(2) Preparation of recombinant DNA

Sau3AI was added to the suspension containing 1 μg of chromosomal DNA obtained in (1) to perform partial digestion. Separately, BamHI was added to 20 μℓ of a solution containing 1 μg of vector pUC19 for digestion. Then, 2 μℓ of 1 M Tris-hydrochloride buffer (pH 8.0) was added thereto and the mixture was treated with alkaline phosphatase at 65°C for an hour. The digested chromosomal DNA and vector DNA described above were purified by the same procedures for phenol extraction and ethanol precipitation as in (1). After 100 ng of purified chromosomal DNA and 20 ng of purified vector DNA were suspended in a solution containing 66 mM Tris-hydrochloride buffer (pH 7.6), 66 mM magnesium chloride, 10 mM DTT and 0.1 mM ATP, 10 units of T4 ligase were added to the suspension. The mixture was allowed to stand at 14°C for 16 hours for the purpose of ligating chromosomal DNA with vector DNA to obtain a recombinant DNA.

(3) Preparation of an Escherichia coli strain with the recombinant DNA introduced therein

Escherichia coli HM70 strain was inoculated in 50 mℓ of LB liquid medium followed by culturing at 37°C for 4 hours. The bacteria harvested by centrifugation at 3000 rpm for 7 minutes were suspended at 0°C in 20 mℓ of 50 mM calcium chloride solution. After the suspension was allowed to stand at 0°C for 20 minutes, the cells were collected by the same centrifugation as described above and suspended at 0°C in 40 mℓ of 50 mM calcium chloride solution. The suspension was mixed with the solution containing the recombinant DNA obtained in (2) and the mixture was allowed to stand at 0°C for 10 minutes. After heat-treating at 42°C for 90 seconds, the mixture was smeared on minimum agar plate medium containing 50 μg/mℓ ampicillin and 5 mM inosine. This plate medium was kept at 30°C for 48 to 72 hours.

(4) Isolation of DNA encoding inosine-guanosine kinase

Several colonies appeared on the plate medium described in (3) in 2 to 3 days. Each of the colonies was cultured at 30°C overnight in LB liquid medium. A part of each culture was smeared on a minimum agar plate medium containing 50 μg/mℓ ampicillin and 5 mM inosine, which was kept at 30°C for 36 to 48 hours. On the second day a colony which grew well, namely, a transformant carrying a recombinant DNA containing the inosine-guanosine kinase gene was selected. The transformant carrying a recombinant DNA containing the inosine-guanosine kinase gene was cultured in LB liquid medium at 30°C overnight. After the cells were harvested, plasmid DNA was isolated by the method described in Maniatis et al. [Molecular Cloning (1982), Cold Spring Harbor Laboratory] and its nucleotide sequence was determined by the dideoxy method [Messing, J., Methods in Enzymology, 101], 20-78 (1983)]. The nucleotide sequence of the structural gene moiety of inosine-guanosine kinase is as shown in Table 5.

The thus obtained recombinant DNA containing the gene encoding inosine-guanosine kinase derived from Escherichia coli HM70 strain was named pBM2. A restriction enzyme map of pBM2 is shown in Fig. 3.

In Fig. 3, part indicated with black bold line is the structural gene encoding inosine-guanosine kinase derived from Escherichia coli HM70 strain. The direction of transcription of the gene is from ClaI site to BglII site in the figure.

(5) Construction of the plasmid which highly expresses inosine-guanosine kinase and its introduction into a microorganism

The structural gene of inosine-guanosine kinase is ligated downstream of tryptophan promoter of Escherichia coli, whereby inosine-guanosine kinase can be expressed in high efficiency.

According to Molecular Cloning (pp. 86-96), plasmid pBM2 obtained in (4) was purified. Using restriction enzymes SmaI (5 units) and KpnI (5 units), 10 μg of pBM2 was fully digested. The resulting digested product was treated with ExoIII nuclease to prepare a mutant in which nucleotides located in the direction of the inserted fragment are deleted. Mutant plasmid pBMΔ14 (Fig. 4) in which the deletion had occurred up to 5' end of the inosine-guanosine kinase structural gene was selected. For construction of the deletion mutant, Deletion Kit for Kilo sequencing (manufactured by Takara, Japan) was used according to the brochure attached. This plasmid pBMΔ14 was digested with PstI and XbaI and a mutant in which nucleotides located to the direction of the inserted fragment were digested by ExoIII nuclease, was prepared in the same way. Thus, mutant plasmid pBMM5 (Fig. 5) in which the deletion had occurred up to 3' end of the inosine-guanosine kinase structural gene was obtained. After 10 μg of pBMM5 was digested with 10 units of restriction enzyme HindIII, the digested end was made to be a blunt end using the DNA blunting kit. Ethanol was added to collect the DNA having a blunt end as a precipitate and this DNA was digested with restriction enzyme SacI. The digested products was isolated by agarose gel electrophoresis and a fragment containing the inosine-guanosine kinase structural gene was recovered from the gel (using DNA Prep manufactured by Asahi Glass Co., Ltd.) The recovered fragment was inserted into SacI-NruI site of vector pTrS30 (Fig. 6) to give plasmid pIKI. Then 10 μg of pIKI was digested with 5 units of ClaI (ClaI site in the inosine-guanosine kinase structural gene does not undergo digestion since it is generally modified by methylation). After the digested product was recovered as a precipitate by addition of ethanol, deletion was introduced at both of the digestion ends using nuclease BAL31. The deletion plasmid was recovered by precipitation by addition of ethanol followed by ligation treatment (ligation kit). The deletion plasmid was used for transformation of MC1000 strain. From the obtained transformants, various deletion plasmids were obtained. From these plasmids, plasmid pIK75 (Fig. 7) in which the inosine-guanosine kinase structural gene had been ligated right downstream of the Shine-Dalgarno sequence derived from the vector was selected. The nucleotide sequence at the junction between the tryptophan promoter and the inosine-guanosine kinase structural gene is shown in Fig. 8. Genetic engineering techniques such as digestion with restriction enzymes, etc. used in this section were performed according to Molecular Cloning, unless otherwise indicated.

(6) Culture of a highly inosine-guanosine kinase-expressed microorganism

When MC1000 strain carrying plasmid pIK75 obtained in (5) [hereafter referred to as MC1000(pIK75)] is cultured, high density culture method [Biotechnol. Bioeng., 17, 227-239 (1975)] is appropriate. MC1000(pIK75) strain was cultured by the high density culture method.

MC1000(pIK75) strain was inoculated into 500 mℓ of seed medium having the following composition followed by culturing at 30°C for 16 hours.

Composition of seed medium: 3.5 g/ℓ KH_2PO_4, 3.5 g/ℓ $(NH_4)_2HPO_4$, 1.0 g/ℓ $MgSO_4 \cdot 7H_20$, 5.0 g/ℓ glucose, 5.0 g/ℓ yeast extract, 3 mℓ/ℓ trace element solution (sterilized at 120°C for 30 minutes by steam)

Composition of trace element solution: 27 g/ℓ $FeCl_3 \cdot 6H_2O$, 2 g/ℓ $ZnCl_2 \cdot 4H_20$, 2 g/ℓ $CoCl_2 \cdot 6H_2O$, 2 g/ℓ $Na_2MoO_4 \cdot 2H_2O$, 1 g/ℓ $CaCl_2 \cdot 2H_2O$, 1 g/ℓ $CuCl_2$, 0.5 g/ℓ H_3BO_3, 100 mℓ/ℓ conc. HCl

Next, 3 ℓ of a fermentation medium (medium obtained by further adding 10 g of $KH_2PO_4 \cdot 3H_2O$ and 5 g of $MgSO_4 \cdot 7H_2O$ to 3 ℓ of seed medium having the composition described above) was charged in a fermentation tank of 7.5 ℓ volume followed by sterilization at 120°C for 30 minutes with steam. After 150 mℓ of 50% (w/v) sterile glucose solution was added to the fermentation tank. 500 mℓ of the seed medium in which MC1000 (pIK75) had been cultured was added.

While adjusting the pH to 6.8 with 5.5 M aqueous ammonia, culture was continued for 24 hours with agitation (6000 rpm) and aeration (3 ℓ/min). In 4 to 6 hours after initiation of culture, a glucose concentration in the culture was reduced to 2.5% or less. From this point, 50% (w/v) glucose solution was continuously fed in the fermentation tank little by little to maintain the glucose concentration in the culture at 2 to 3%. (The culture was centrifuged and the cells recovered as precipitates were stored at -20°C. The cells were used in the following (7) and (9). Furthermore the medium after completion of the culture was frozen at -20°C and thawed at 30°C immediately before use in the following (10)).

(7) Purification of inosine-guanosine kinase

After 6 g of the -20°C-stored cells which had been obtained in (6) was suspended in 24 mℓ of purification buffer [20% glycerol, 50 mM Tris-hydrochloride buffer (pH 8) and 5 mM β-mercactoethanol], the suspension was disrupted with a homogenizer (manufactured by Brown Biotech Co., Ltd., glass bead diameter of 0.1 mm). The homogenate was

centrifuged to give about 20 mℓ of the supernatant. Protamine sulfate was added to the supernatant in a final concentration of 0.4% followed by centrifugation. Thus high molecular nucleic acid components were removed as precipitates. The resulting supernatant was passed through Sephadex G-50 column which had been previously equilibrated with the purification buffer. The column was eluted with the purification buffer to give about 30 mℓ of desalted active fraction. After the fraction was passed through DEAE Sepharose column which had been previously equilibrated with the purification buffer, 60 mℓ of the purification buffer containing 0.1 M NaCl was added thereto. Then the desired enzyme was eluted with 200 mℓ of the purification buffer having an NaCl linear concentration gradient of 0.1 M to 0.6 M. From the active fractions eluted, 3 mℓ of the fraction having the highest enzyme concentration was collected. This active fraction was passed through 'Sephacryl S-200 column and eluted with the purification buffer containing 0.1 M NaCl. By gel filtration, the active fraction was collected. Finally 10 mℓ of solution purified enzyme (1.4 µg protein/mℓ) was obtained. This purified enzyme was analyzed by SDS-polyacrylamide electrophoresis and no impurity was detected. The enzyme is stably stored at -20°C in the purification buffer containing 0.1 M NaCl.

(8) Culture of a microorganism capable of regenerating ATP

Brevibacterium ammoniagenes KY13761 strain was cultured at 30°C for 2 days on a seed agar medium plate prepared by adding 25 g/ℓ agar to a seed medium having the composition described in Table 7. The obtained cells were inoculated into 30 mℓ of seed medium contained in an Erlenmeyer's flask of 250 mℓ volume followed by shaking culture at 30°C for 24 hours.

The 30 mℓ of the resulting culture was inoculated into 3 ℓ of seed medium having the composition described in Table 7, in a fermentation tank of 5 ℓ volume. While adjusting pH to 6.8 with 5.5 M aqueous ammonia, culture was continued for 24 hours with agitation at 600 rpm and aeration of 3 ℓ/min.

300 mℓ of the obtained culture was inoculated into 3 ℓ of fermentation medium having a composition described in Table 7,in a fermentation tank of 5 ℓ volume. While adjusting pH to 6.8 with 5.5 M aqueous ammonia, culture was continued at 32°C for 42 hours with agitation at 600 rpm and aeration of 3 ℓ/min. [The medium was centrifuged and the cells recovered as precipitates were stored at -20°C. The cells were used in the following (9). Furthermore the medium (containing about 30 g/ℓ of inosine) after completion of the culture was directly frozen at -20°C and thawed at 30°C immediately before use in the following (10)].

Table 7

Composition (g/ℓ)	Seed Medium	Fermentation Medium
Glucose	50	150
KH_2PO_4	1	10
K_2HPO_4	3	10
$MgSO_4 \cdot 7H_2O$	1	10
$CaCl_2 \cdot 2H_2O$	0.1	0.1
$FeSO_4 \cdot 7H_2O$	0.01	0.01
$ZnSO_4 \cdot 7H_2O$	0.001	0.001
$MnSO_4 \cdot 4\text{-}6H_2O$	0.004	0.004
L-Cystein·HCl	0.02	0.02

Thiamine	0.005	0.005
Ca-D-pantothenate	0.01	0.01
Nicotinic aicd	none	0.005
Biotin	30 µg/ℓ	30 µg/ℓ
Urea	5	2
$(NH_4)_2SO_4$	5	none
Meat extract	none	10
Polypeptone	10	none
Yeast extract	10	none
Adenine	0.3	0.2
pH	7.2	8.3

[used after steam sterilization (120°C, 30 minutes)]

(9) Production of 5'-nucleotides from nucleosides by resting cell reaction

While maintaining at 32°C by strongly stirring 20 mℓ of a solution having composition shown in Table 8, reaction was carried out for 24 hours, while keeping pH at 7.2 using 4 N NaOH. A small amount of the reaction mixture was subjected to sampling intermittently to determine concentrations of phosphoric acid, inosine and 5'-IMP in the mixture. For quantitative assay for phosphoric acid, Phospho B-test WAKO (manufactured by Wako Pure Chemical Industry Co., Ltd.) was used and a difference between measurement data every time and initial addition was complemented to keep the concentration of phosphate ion constant by adding monopotassium phosphate. Concentrations of inosine and 5'-IMP were quantitatively determined by HPLC according to a modification of the method described hereinbefore. About 100 g/ℓ of 5'-IMP (calculated as disodium 7.5 hydrate) was produced from about 50 g/ℓ of initial inosine 24 hours after starting the reaction. In this case, the molar conversion rate was 90% or more. When guanosine was added instead of inosine, 5'-GMP was produced in a molar conversion rate of 90% or more with similar time course.

Table 8

Composition of reaction solution	
KY13761 strain	200 g wet cell weight/ℓ
MC1000 (pIK75) strain	20 g wet cell weight/ℓ
Inosine	50 g/ℓ
Monopotassium phosphate	20 g/ℓ
Glucose	30 g/ℓ
Magnesium sulfate	5 g/ℓ
Xylene	10 mℓ/ℓ
Nymeen S-215	4 g/ℓ
Phytic acid	5 g/ℓ

(10) Production of 5'-IMP from inosine using inosine fermentation medium

After completion of fermentation, about 30 g/ℓ of inosine was accumulated in the medium obtained in (8) above. As indicated in Table 9, 20 mℓ of a reaction mixture containing this medium was prepared. In a manner similar to (9), while keeping at 32°C with vigorously stirring and also keeping pH at 7.2 with 4 N NaOH, a reaction was carried out for 13 hours. During the course of reaction, monopotassium phosphate was added as in (9). From 23.5 g/ℓ of initial inosine, 46 g/ℓ of 5'-IMP was produced and accumulated. Also in this case, its molar conversion rate was 90% or more, as in (9).

Table 9

Composition of reaction solution

Inosine culture broth (containing KY13761 cells)	15.7 mℓ
MC1000(pIK75) culture broth	1.82 mℓ
Monopotassium phosphate	0.4 g
Glucose	0.7 g
Magnesium sulfate	0.1 g
Xylene	0.2 mℓ
Nymeen S-215	80 mg
phytic acid	100 mg

(Distilled water is added to the foregoing components until 20 mℓ).

Example 2

(1) Isolation of DNA

Purified chromosomal DNA was obtained in a manner similar to Example 1 (1) except for using Escherichia coli W3110 strain in place of Escherichia coli HM70 strain. The chromosomal DNA was suspended in 10 mM Tris-hydrochloride buffer containing 1 mM EDTA.

(2) Preparation of recombinant DNA

A recombinant DNA was obtained in a manner similar to Example 1 (2) except for using the suspension containing 1 µg of chromosomal DNA obtained in Example 2 (1) in place of the suspension obtained in Example 1 (1).

(3) Preparation of an Escherichia coli strain with the recombinant DNA introduced therein

Escherichia coli DHI strain was inoculated into 50 mℓ of LB liquid medium followed by culturing at 37°C for 4 hours. The bacteria harvested by centrifugation at 3000 rpm for 7 minutes were suspended at 0°C in 20 mℓ of 50 mM calcium chloride solution. The suspension was allowed to stand at 0°C for 20 minutes, the cells were collected by the same centrifugation as described above and suspended at 0°C in 40 mℓ of 50 mM calcium chloride solution. The suspension was mixed with the solution containing recombinant DNA obtained in Example 2 (2) and the mixture was allowed to stand at 0°C for 10 minutes. After heat-treating at 42°C for 90 seconds, the mixture was smeared on LB agar medium plate (LB liquid medium containing 1.5% agar) supplemented with 50 µg/mℓ of ampicillin, 0.1 mM isopropylthiogalactoside (IPTG) and 0.004% of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.

The resulting white colony was cultured at 30°C overnight in 10 mℓ of LB liquid medium and the cells were isolated by centrifugation. These isolated cells were stored at -20°C and their inosine-guanosine kinase activity was determined by the method shown below.

(4) Isolation of DNA encoding inosine-guanosine kinase

Escherichia coli cells containing the recombinant DNA collected in Example 2 (3) were suspended (cell concentration was 100 g wet cell weight/ml) in 100 mM Tris-hydrochloride buffer (pH 8.0) containing 10 mM ATP, 10 mM inosine and 5 mM magnesium sulfate. Xylene was added to the suspension in a concentration of 10 mℓ/ℓ. After thoroughly stirring, the mixture was allowed to stand at 30°C for an hour. The reaction solution was analyzed by HPLC and the amount of 5'-IMP in the reaction solution was quantitatively determined. Almost all transformants did not produce 5'-IMP but 5'-IMP-producing bacteria were obtained in a proportion of 1 out of 50,000 samples. The thus obtained 5'-IMP-pro-

ducing bacteria are transformants carrying a recombinant DNA containing an inosine-guanosine kinase gene.

The obtained transformants were cultured at 30°C overnight in LB liquid medium and the cells were collected. Thereafter plasmid DNA was isolated by the method described in Molecular cloning and its nucleotide sequence was determined by the dideoxy method. The nucleotide sequence of the structural gene moiety of inosine-guanosine kinase is as shown in Table 5.

The thus obtained recombinant DNA containing the gene encoding inosine-guanosine kinase derived from Escherichia coli W3110 strain was named pBMI. A restriction enzyme map of pBMI is shown in Fig. 9.

(5) Preparation of 5'-IMP

Transformant Escherichia coli HMI (FERM BP-2669) transformed with recombinant DNA pBMI obtained in Example 2 (4) was cultured at 30°C for 16 hours in 400 mℓ of LB liquid medium (containing 50 µg/ml of ampicillin). The medium was then centrifuged to recover the cells. For control, the culture cells of Escherichia coli DHI/pUC19 containig vector pUC19 alone were obtained in a manner similar to the above. After 20 mℓ of a solution of Table 10 containing the obtained cells was reacted at 30°C for an hour, the amount of 5'-IMP produced in the solution was quantitatively determined by HPLC and found to be 5 mg. When the cells from the strain carrying the vector for control were used, no 5'-IMP was detected in the solution.

Table 10

Composition of reaction solution	
Cell	100.g wet cell weight/ℓ
Inosine	10 mM
ATP	10 mM
Tris-hydrochloride buffer (pH 8.0)	100 mM
Magnesium sulfate	5 mM
Xylene	10 mℓ/ℓ

(6) Preparation of 5'-GMP

Transformant Escherichia coli HMI carrying recombinant DNA pBMI obtained in Example 2 (4) was cultured at 30°C for 16 hours in 400 mℓ of LB liquid medium (containing 50 µg/mℓ ampicillin). The medium was then centrifuged to recover the cells. For control, the culture cells of Escherichia coli DHI/pUCI9 containing vector pUCI9 alone were obtained in a manner similar to the above. After 20 mℓ of a solution of Table 11 containing the obtained cells was incubated at 30°C for an hour, the amount of 5'-GMP produced in the solution was quantitatively determined by HPLC and found to be 2 mg. When the cell of the strain carrying the vector for control was used, no 5'-GMP was detected in the solution.

Table 11

Composition of reaction solution	
Cell	100 g wet cell weight/ℓ
Guanosine	10 mM
ATP	10 mM
Tris-hydrochloride buffer (pH 8.0)	100 mM
Magnesium sulfate	5 mM
Xylene	10 mℓ/ℓ

(7) Amplification of activity by genetic engineering

By ligating SD sequence and strong promoter sequence upstream the inosine-guanosine kinase structural gene by genetic engineering technique. expression of inosine-guanosine kinase can be amplified. The amplification can be made as follows.

After 20 µℓ of a solution containing 1 µg of plasmid pBMI obtained in Example 2 (4) was fully digested with 10 units of BamHI, 10 units of SacI and 10 units of ScaI, 2.8 kb fragment containing the inosine-guanosine kinase structural gene was isolated and purified by agarose gel electrophoresis (Molecular Cloning). After the purified fragment was digested with BAL31 nuclease at 37°C for 10 minutes, phenol extraction and ethanol precipitation were performed to obtain a DNA fragment having deletion at the end. Separately, 10 units of SmaI was added to 20 µℓ of a solution

containing 1 μg of vector pUC19 for full digestion. Then 2 μℓ of 1 M Tris-hydrochloride buffer (pH 8.0) was added and further 5 units of alkaline phosphatase was added thereto followed by reaction at 65°C for an hour. The purified inosine-guanosine kinase gene-containing DNA fragment (100 ng) and 20 ng of alkaline phosphatase-treated vector DNA were suspended in a solution containing 66 mM Tris-hydrochloride buffer (pH 7.6), 66 mM magnesium chloride, 10 mM DTT and 0.1 mM ATP. To the suspension were added 10 units of T4 ligase. The mixture was reacted at 14°C for 16 hours to ligate both DNAs, thereby to obtain a recombinant DNA. The recombinant DNA was introduced into Escherichia coli DHI strain in a manner similar to Example 2 (3). The obtained transformants were cultured in a manner similar to Example 2 (3). The inosine-guanosine kinase activity was determined in a manner similar to Example 2 (4) and a strain having a high 5'-IMP productivity was selected. The strain was named Escherichia coli 3M100 strain. In recombinant plasmid carried by Escherichia coli BM100 strain, lactose promoter sequence was ligated upstream the inosine-guanosine kinase structural gene in a correct direction.

(8) Preparation of 5'-IMP

Escherichia coli BM100 strain was cultured in 400 mℓ of LB liquid medium (containing 50 μg/mℓ ampicillin) at 30°C for 16 hours. Centrifugation gave the cells. After 20 mℓ of a solution given by Table 12 containing the obtained cells and a strain having ATP regenerating activity (Japanese Published Unexamined Patent Application No. 74595/86) was incubated at 30°C for 20 hours at pH of 7.6, the amount of 5'-IMP produced in the solution was quantitatively assayed and found to be 25 g/ℓ (calculated as the hydrate). Also in the case of using a strain carrying a recombinant DNA containing a DNA molecule, in which part of the structural gene, i.e., the DNA sequence coding for the amino-acid sequence subsequent to the 8th Leu from the C terminal is modified to code for the following amino acid sequence:

GlyMetGlnAlaGlyThrGluLeuGluPheThrGlyArgArgPheThrThrSer,

a similar titer can be obtained.

Table 12

Composition of reaction solution	
Cells of Brevibacterium ammoniagenes ATCC21477 (ATP-regenerating cell)	200 g wet cell weight/ℓ
Inosine-guanosine kinase amplified Escherichia coli	25 g wet cell weight/ℓ
Inosine	12.5 g/ℓ
Monopotassium phosphate	20 g/ℓ
Glucose	50 g/ℓ
Magnesium sulfate	5 g/ℓ
Xylene	10 mℓ/ℓ

Industrial Applicability

According to the present invention, inosine-guanosine kinase derived from a microorganism belonging to the genus Escherichia can be provided. In addition, clarification of various properties of the enzyme for the first time has led to industrial utilization thereof. Furthermore, DNA encoding the enzyme has been isolated. Using as a catalyst a microorganism containing the recombinant DNA obtained by introducing this DNA into a vector, 5'-nucleotides, e.g., 5'-IMP or 5'-GMP, can be produced from nucleosides, e.g., inosine or guanosine, in a high conversion rate.

Claims

Claims for the following Contracting States : AT, BE, CH, LI, DE, FR, GB, IT, LU, NL, SE

1. A purified inosine-guanosine kinase having the following physicochemical properties:

(1) Action

The enzyme has an action of forming adenosine diphosphate (ADP) and 5'-inosinic acid (5'-IMP) from adenosine triphosphate (ATP) and inosine, an action of forming deoxyadenosine diphosphate (dADP) and 5'-IMP from deoxyadenosine triphosphate (dATP) and inosine, an action of forming ADP and 5'-guanylic acid (5'-GMP) from ATP and guanosine, and an action of forming dADP and 5'-GMP from dATP and guanosine.

(2) Optimum pH

6.9 - 8.2

(3) pH stability

The enzyme is stably kept in a pH range of 6.6 to 9.0 by treatment at 4°C for 16 hours. The addition of KCl ameliorates stability during storage.

(4) Optimum temperature

25 - 40°C

(5) Temperature stability

Stable up to 40°C by treatment at pH of 8.0 for 15 minutes

(6) Substrate specificity

Phosphoric acid at the γ-position of ATP or dATP used as phosphate donors is transfered to inosine or guanosine used as phosphate acceptors.

(7) Inhibitors

Metal ions of Co^{2+}, Cu^{2+} and Zn^{2+}

(8) Activation

The enzyme requires one of the two ion groups: one is K^+ and Mg^{2+}, and the other K^+ and Mn^{2+}.

(9) Km value

Km value in the reaction solution composed of 2 mM ATP, 10 mM $MgSO_4$, 300 mM KCl and 0.1 M HEPES buffer (pH 7.2) is 2.1 mM for inosine and 6.1 μM for guanosine.

(10) Molecular weight

Molecular weight deduced from the amino acid sequence is about 48,400 daltons; according to measurement by SDS-polyacrylamide electrophoresis, molecular weight is about 43,000 daltons.

2. A purified inosine-guanosine kinase represented by the following amino acid sequence:

```
  1  MetLysPheProGlyLysArgLysSerLysHisTyrPheProValAsnAlaArgAspPro
 21  LeuLeuGlnGlnPheGlnProGluAsnGluThrSerAlaAlaTrpValValGlyIleAsp
 41  GlnThrLeuValAspIleGluAlaLysValAspAspGluPheIleGluArgTyrGlyLeu
 61  SerAlaGlyHisSerLeuValIleGluAspAspValAlaGluAlaLeuTyrGlnGluLeu
 81  LysGlnLysAsnLeuIleThrHisGlnPheAlaGlyGlyThrIleGlyAsnThrMetHis
101  AsnTyrSerValLeuAlaAspAspArgSerValLeuLeuGlyValMetCysSerAsnIle
121  GluIleGlySerTyrAlaTyrArgTyrLeuCysAsnThrSerSerArgThrAspLeuAsn
141  TyrLeuGlnGlyValAspGlyProIleGlyArgCysPheThrLeuIleGlyGluSerGly
161  GluArgThrPheAlaIleSerProGlyHisMetAsnGlnLeuArgAlaGluSerIlePro
181  GluAspValIleAlaGlyAlaSerAlaLeuValLeuThrSerTyrLeuValArgCysLys
201  ProGlyGluProMetProGluAlaThrMetLysAlaIleGluTyrAlaLysLysTyrAsn
221  ValProValValLeuThrLeuGlyThrLysPheValIleAlaGluAsnProGlnTrpTrp
241  GlnGlnPheLeuLysAspHisValSerIleLeuAlaMetAsnGluAspGluAlaGluAla
261  LeuThrGlyGluSerAspProLeuLeuAlaSerAspLysAlaLeuAspTrpValAspLeu
281  ValLeuCysThrAlaGlyProIleGlyLeuTyrMetAlaGlyPheThrGluAspGluAla
301  LysArgLysThrGlnHisProLeuLeuProGlyAlaIleAlaGluPheAsnGlnTyrGlu
321  PheSerArgAlaMetArgHisLysAspCysGlnAsnProLeuArgValTyrSerHisIle
341  AlaProTyrMetGlyGlyProGluLysIleMetAsnThrAsnGlyAlaGlyAspGlyAla
361  LeuAlaAlaLeuLeuHisAspIleThrAlaAsnSerTyrHisArgSerAsnValProAsn
381  SerSerLysHisLysPheThrTrpLeuThrTyrSerSerLeuAlaGlnValCysLysTyr
401  AlaAsnArgValSerTyrGlnValLeuAsnGlnHisSerProArgLeuThrArgGlyLeu
421  ProGluArgGluAspSerLeuGluGluSerTyrTrpAspArg
```

3. A gene encoding an inosine-guanosine kinase having the amino acid sequence as defined in claim 2.

4. The gene as claimed in claim 3. wherein said gene is shown by the following nucleotide sequence:

```
   1  ATGAAATTTC CCGGTAAACG TAAATCCAAA CATTACTTCC CCGTAAACGC
  51  ACGCGATCCG CTGCTTCAGC AATTCCAGCC AGAAAACGAA ACCAGCGCTG
 101  CCTGGGTAGT GGGTATCGAT CAAACGCTGG TCGATATTGA AGCGAAAGTG
 151  GATGATGAAT TTATTGAGCG TTATGGATTA AGCGCCGGGC ATTCACTGGT
 201  GATTGAGGAT GATGTAGCCG AAGCGCTTTA TCAGGAACTA AAACAGAAAA
 251  ACCTGATTAC CCATCAGTTT GCGGGTGGCA CCATTGGTAA CACCATGCAC
 301  AACTACTCGG TGCTCGCGGA CGACCGTTCG GTGCTGCTGG GCGTCATGTG
 351  CAGCAATATT GAAATTGGCA GTTATGCCTA TCGTTACCTG TGTAACACTT
 401  CCAGCCGTAC CGATCTTAAC TATCTACAAG GCGTGGATGG CCCGATTGGT
 451  CGTTGCTTTA CGCTGATTGG CGAGTCCGGG GAACGTACCT TTGCTATCAG
 501  TCCAGGCCAC ATGAACCAGC TGCGGGCTGA AAGCATTCCG GAAGATGTGA
 551  TTGCCGGAGC CTCGGCACTG GTTCTCACCT CATATCTGGT GCGTTGCAAG
 601  CCGGGTGAAC CCATGCCGGA AGCAACCATG AAAGCCATTG AGTACGCGAA
 651  GAAATATAAC GTACCGGTGG TGCTGACGCT GGGCACCAAG TTTGTCATTG
 701  CCGAGAATCC GCAGTGGTGG CAGCAATTCC TCAAAGATCA CGTCTCTATC
 751  CTTGCGATGA ACGAAGATGA AGCCGAAGCG TTGACCGGAG AAAGCGATCC
 801  GTTGTTGGCA TCTGACAAGG CGCTGGACTG GGTAGATCTG GTGCTGTGCA
 851  CCGCCGGGCC AATCGGCTTG TATATGGCGG GCTTTACCGA AGACGAAGCG
 901  AAACGTAAAA CCCAGCATCC GCTGCTGCCG GGCGCTATAG CGGAATTCAA
 951  CCAGTATGAG TTTAGCCGCG CCATGCGCCA CAAGGATTGC CAGAATCCGC
1001  TGCGTGTATA TTCGCACATT GCGCCGTACA TGGGCGGGCC GGAAAAAATC
1051  ATGAACACTA ATGGAGCGGG GGATGGCGCA TTGGCAGCGT TGCTGCATGA
1101  CATTACCGCC AACAGCTACC ATCGTAGCAA CGTACCAAAC TCCAGCAAAC
1151  ATAAATTCAC CTGGTTAACT TATTCATCGT TAGCGCAGGT GTGTAAATAT
1201  GCTAACCGTG TGAGCTATCA GGTACTGAAC CAGCATTCAC CTCGTTTAAC
1251  GCGCGGCTTG CCGGAGCGTG AAGACAGCCT GGAAGAGTCT TACTGGGATC
1301  GT
```

5. The gene as claimed in claim 3 or 4, wherein said gene is derived from Escherichia coli.

6. A recombinant DNA obtained by inserting into a vector a DNA fragment containing a gene as defined by any one of claims 3 to 5.

7. A microorganism carrying a recombinant DNA as defined by claim 6.

8. The microorganism as claimed in claim 7, wherein said microorganism is Escherichia coli.

9. A process for preparing an inosine-guanosine kinase which comprises culturing in a medium a microorganism belonging to the genus Escherichia and carrying a recombinant DNA as defined in claim 6 to produce and accumulate the inosine-guanosine kinase in the cultured cells and collecting the inosine-guanosine kinase from the culture.

10. A process for preparing 5' -IMP or 5' -GMP which comprises reacting inosine or guanosine with a phosphate donor in an aqueous medium in the presence of an enzyme source which is (1) a purified inosine-guanosine kinase as defined in claim 1 or 2 or (2) a cell, a culture broth or a treated matter of the microorganism as defined by claim 7 to produce and accumulate 5' -IMP or 5' -GMP in the reaction mixture, and collecting 5'-IMP or 5' -GMP from the reaction mixture.

11. The process for preparing 5' -IMP or 5' -GMP as claimed in claim 10, wherein said phosphate donor is ATP or dATP.

12. The process for preparing 5' -IMP or 5' -GMP as claimed in claim 10, wherein said microorganism is a microorganism belonging to the genus Escherichia.

Claims for the following Contracting State : ES

1. A process for preparing an inosine-guanosine kinase which comprises culturing in a medium a microorganism belonging to the genus Escherichia and carrying a recombinant DNA obtained by inserting into a vector a DNA fragment containing a gene encoding an inosine-guanosine kinase having following amino acid sequence.

```
  1  MetLysPheProGlyLysArgLysSerLysHisTyrPheProValAsnAlaArgAspPro
 21  LeuLeuGlnGlnPheGlnProGluAsnGluThrSerAlaAlaTrpValValGlyIleAsp
 41  GlnThrLeuValAspIleGluAlaLysValAspAspGluPheIleGluArgTyrGlyLeu
 61  SerAlaGlyHisSerLeuValIleGluAspAspValAlaGluAlaLeuTyrGlnGluLeu
 81  LysGlnLysAsnLeuIleThrHisGlnPheAlaGlyGlyThrIleGlyAsnThrMetHis
101  AsnTyrSerValLeuAlaAspAspArgSerValLeuLeuGlyValMetCysSerAsnIle
121  GluIleGlySerTyrAlaTyrArgTyrLeuCysAsnThrSerSerArgThrAspLeuAsn
141  TyrLeuGlnGlyValAspGlyProIleGlyArgCysPheThrLeuIleGlyGluSerGly
161  GluArgThrPheAlaIleSerProGlyHisMetAsnGlnLeuArgAlaGluSerIlePro
181  GluAspValIleAlaGlyAlaSerAlaLeuValLeuThrSerTyrLeuValArgCysLys
201  ProGlyGluProMetProGluAlaThrMetLysAlaIleGluTyrAlaLysLysTyrAsn
221  ValProValValLeuThrLeuGlyThrLysPheValIleAlaGluAsnProGlnTrpTrp
241  GlnGlnPheLeuLysAspHisValSerIleLeuAlaMetAsnGluAspGluAlaGluAla
261  LeuThrGlyGluSerAspProLeuLeuAlaSerAspLysAlaLeuAspTrpValAspLeu
281  ValLeuCysThrAlaGlyProIleGlyLeuTyrMetAlaGlyPheThrGluAspGluAla
301  LysArgLysThrGlnHisProLeuLeuProGlyAlaIleAlaGluPheAsnGlnTyrGlu
321  PheSerArgAlaMetArgHisLysAspCysGlnAsnProLeuArgValTyrSerHisIle
341  AlaProTyrMetGlyGlyProGluLysIleMetAsnThrAsnGlyAlaGlyAspGlyAla
361  LeuAlaAlaLeuLeuHisAspIleThrAlaAsnSerTyrHisArgSerAsnValProAsn
381  SerSerLysHisLysPheThrTrpLeuThrTyrSerSerLeuAlaGlnValCysLysTyr
401  AlaAsnArgValSerTyrGlnValLeuAsnGlnHisSerProArgLeuThrArgGlyLeu
421  ProGluArgGluAspSerLeuGluGluSerTyrTrpAspArg
```

2. The process according to claim 1 characterized in that an inosine-guanosine kinase is prepared having the following physicochemical properties :

(1) Action

The enzyme has an action of forming adenosine diphosphate (ADP) and 5'-inosinic acid (5'-IMP) from adenosine triphosphate (ATP) and inosine. an action of forming deoxyadenosine diphosphate (dADP) and 5'-IMP from deoxyadenosine triphosphate (dATP) and inosine. an action of forming ADP and 5'-guanylic acid (5'-GMP) from AT? and guanosine. and an action of forming dADP and 5'-GMP from dATP and guanosine.

(2) Optimum pH

6.9 - 8.2

(3) pH stability

The enzyme is stably kept in a pH range of 6.6 to 9.0 by treatment at 4°C for 16 hours. The addition of KCl

ameliorates stability during storage.

(4) Optimum temperature

25 - 40°C

(5) Temperature stability

Stable up to 40°C by treatment at pH of 8.0 for 15 minutes

(6) Substrate specificity

Phosphocic acid at the γ-position of ATP or dATP used as a phosphate donors is transfered to inosine or guanosine used as phophate acceptors,

(7) Inhibitor

Metal ions of Co^{2+}, Cu^{2+} and Zn^{2+}

(8) Activation

The enzyme requires one of the two ion groups: one is K^+ and Mg^{2+}, and the other K^+ and Mn^{2+}.

(9) Km value

Km value in the reaction solution composed of 2 mM ATP, 10 mM $MgSO_4$, 300 mM KCl and 0.1 M HEPES buffer (pH 7.2) is 2.1 mM for inosine and 6.1 μM for guanosine.

(10) Molecular weight

Molecular weight deduced from the amino acid sequence is about 48,400 daltons; according to measurement by SDS-polyacrylamide electrophoresis, molecular weight is about 43,000 daltons.

3. The process according to claim 1 characterized in that the gene contained in the DNA fragment inserted into the vector has following nucleotide sequence.

```
   1  ATGAAATTTC CCGGTAAACG TAAATCCAAA CATTACTTCC CCGTAAACGC
  51  ACGCGATCCG CTGCTTCAGC AATTCCAGCC AGAAAACGAA ACCAGCGCTG
 101  CCTGGGTAGT GGGTATCGAT CAAACGCTGG TCGATATTGA AGCGAAAGTG
 151  GATGATGAAT TTATTGAGCG TTATGGATTA AGCGCCGGGC ATTCACTGGT
 201  GATTGAGGAT GATGTAGCCG AAGCGCTTTA TCAGGAACTA AAACAGAAAA
 251  ACCTGATTAC CCATCAGTTT GCGGGTGGCA CCATTGGTAA CACCATGCAC
 301  AACTACTCGG TGCTCGCGGA CGACCGTTCG GTGCTGCTGG GCGTCATGTG
 351  CAGCAATATT GAAATTGGCA GTTATGCCTA TCGTTACCTG TGTAACACTT
 401  CCAGCCGTAC CGATCTTAAC TATCTACAAG GCGTGGATGG CCCGATTGGT
 451  CGTTGCTTTA CGCTGATTGG CGAGTCCGGG GAACGTACCT TTGCTATCAG
 501  TCCAGGCCAC ATGAACCAGC TGCGGGCTGA AAGCATTCCG GAAGATGTGA
 551  TTGCCGGAGC CTCGGCACTG GTTCTCACCT CATATCTGGT GCGTTGCAAG
 601  CCGGGTGAAC CCATGCCCGA AGCAACCATG AAAGCCATTG AGTACGCGAA
 651  GAAATATAAC GTACCGGTGG TGCTGACGCT GGGCACCAAG TTTGTCATTG
 701  CCGAGAATCC GCAGTGGTGG CAGCAATTCC TCAAAGATCA CGTCTCTATC
 751  CTTGCGATGA ACGAAGATGA AGCCGAAGCG TTGACCGGAG AAAGCGATCC
 801  GTTGTTGGCA TCTGACAAGG CGCTGGACTG GGTAGATCTG GTGCTGTGCA
 851  CCGCCGGGCC AATCGGCTTG TATATGGCGG GCTTTACCGA AGACGAAGCG
 901  AAACGTAAAA CCCAGCATCC GCTGCTGCCG GGCGCTATAG CGGAATTCAA
 951  CCAGTATGAG TTTAGCCGCG CCATGCGCCA CAAGGATTGC CAGAATCCGC
1001  TGCGTGTATA TTCGCACATT GCGCCGTACA TGGGCGGGCC GGAAAAAATC
1051  ATGAACACTA ATGGAGCGGG GGATGGCGCA TTGGCAGCGT TGCTGCATGA
1101  CATTACCGCC AACAGCTACC ATCGTAGCAA CGTACCAAAC TCCAGCAAAC
1151  ATAAATTCAC CTGGTTAACT TATTCATCGT TAGCGCAGGT GTGTAAATAT
1201  GCTAACCGTG TGAGCTATCA GGTACTGAAC CAGCATTCAC CTCGTTTAAC
1251  GCGCGGCTTG CCGGAGCGTG AAGACAGCCT GGAAGAGTCT TACTGGGATC
1301  GT
```

4. A process for preparing a gene encoding an inosine-guanosine kinase comprising the steps of cloning and isolating

a gene encoding an inosine guanosine kinase having the amino acid sequence as defined in claim 1.

5. The process according to claim 4 characterized in that the gene cloned and isolated is shown by the following nucleotide sequence :

```
   1  ATGAAATTTC CCGGTAAACG TAAATCCAAA CATTACTTCC CCGTAAACGC
  51  ACGCGATCCG CTGCTTCAGC AATTCCAGCC AGAAAACGAA ACCAGCGCTG
 101  CCTGGGTAGT GGGTATCGAT CAAACGCTGG TCGATATTGA AGCGAAAGTG
 151  GATGATGAAT TTATTGAGCG TTATGGATTA AGCGCCGGGC ATTCACTGGT
 201  GATTGAGGAT GATGTAGCCG AAGCGCTTTA TCAGGAACTA AAACAGAAAA
 251  ACCTGATTAC CCATCAGTTT GCGGGTGGCA CCATTGGTAA CACCATGCAC
 301  AACTACTCGG TGCTCGCGGA CGACCGTTCG GTGCTGCTGG GCGTCATGTG
 351  CAGCAATATT GAAATTGGCA GTTATGCCTA TCGTTACCTG TGTAACACTT
 401  CCAGCCGTAC CGATCTTAAC TATCTACAAG GCGTGGATGG CCCGATTGGT
 451  CGTTGCTTTA CGCTGATTGG CGAGTCCGGG GAACGTACCT TTGCTATCAG
 501  TCCAGGCCAC ATGAACCAGC TGCGGGCTGA AAGCATTCCG GAAGATGTGA
 551  TTGCCGGAGC CTCGGCACTG GTTCTCACCT CATATCTGGT GCGTTGCAAG
 601  CCGGGTGAAC CCATGCCGGA AGCAACCATG AAAGCCATTG AGTACGCGAA
 651  GAAATATAAC GTACCGGTGG TGCTGACGCT GGGCACCAAG TTTGTCATTG
 701  CCGAGAATCC GCAGTGGTGG CAGCAATTCC TCAAAGATCA CGTCTCTATC
 751  CTTGCGATGA ACGAAGATGA AGCCGAAGCG TTGACCGGAG AAAGCGATCC
 801  GTTGTTGGCA TCTGACAAGG CGCTGGACTG GGTAGATCTG GTGCTGTGCA
 851  CCGCCGGGCC AATCGGCTTG TATATGGCGG GCTTTACCGA AGACGAAGCG
 901  AAACGTAAAA CCCAGCATCC GCTGCTGCCG GGCGCTATAG CGGAATTCAA
 951  CCAGTATGAG TTTAGCCGCG CCATGCGCCA CAAGGATTGC CAGAATCCGC
1001  TGCGTGTATA TTCGCACATT GCGCCGTACA TGGGCGGGCC GGAAAAAATC
1051  ATGAACACTA ATGGAGCGGG GGATGCGCGA TTGGCAGCGT TGCTGCATGA
1101  CATTACCGCC AACAGGTACC ATCGTAGCAA CGTACCAAAC TCCAGCAAAC
1151  ATAAATTCAC CTGGTTAACT TATTCATCGT TACCGCAGGT GTGTAAATAT
1201  GCTAACCGTG TGAGCTATCA GGTACTGAAC CAGCATTCAC CTCGTTTAAC
1251  GCGCGGCTTG CCGGAGCGTG AAGACAGCCT GGAAGAGTCT TACTGGGATC
1301  GT
```

6. The process claimed in claim 4 or 5, wherein said gene is derived from Eschericia coli.

7. A process for producing a recombinant DNA comprising the steps of inserting into a vector a DNA fragment containing a gene as prepared according to anyone of claims 4 to 6.

8. A process for preparing 5' -IMP or 5' -GMP which comprises reacting inosine or guanosine with a phosphate donor in an aqueous medium in the presence of an enzyme source which is (1) an inosine-guanosine kinase as defined in claim 1 or (2) a cell, a culture broth or a treated matter of the microorganism carrying a recombinant DNA as defined in claim 7 to produce and accumulate 5' -IMP or 5' -GMP in the reaction mixture, and collecting 5'-IMP or 5' -GMP from the reaction mixture.

9. The process for preparing 5'-IMP or 5' -GMP as claimed in claim 8, wherein said phosphate donor is ATP or dATP.

10. The process for preparing 5' -IMP or 5'-GMP as claimed in claim 8, vherein said microorganism is a microorganism belonging to the genus Escherichia.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, LI, DE, FR, GB, IT, LU, NL, SE

1. Gereinigte Inosin-Guanosin-Kinase mit den folgenden physikalisch-chemischen Eigenschaften:

(1) Wirkung
Das Enzym katalysiert die Bildung von Adenosindiphosphat (ADP) und 5'-Inosinsäure (5'-IMP) aus Adenosintriphosphat (ATP) und Inosin, die Bildung von Desoxyadenosindiphosphat (dADP) und 5'-IMP aus Desoxyadenosintriphosphat (dATP) und Inosin, die Bildung von ADP und 5'-Guanylsäure (5'-GMP) aus ATP und Guanosin und die Bildung von dADP und 5'-GMP aus dATP und Guanosin,
(2) pH-Optimum
6,9 - 8,2,
(3) pH-Stabilität
das Enzym ist stabil in einem pH-Bereich von 6,6 bis 9,0 bei einer 16stündigen Behandlung bei 4°C, die Zugabe von KCl verbessert die Stabilität während der Lagerung,
(4) Temperaturoptimum
25-40°C,
(5) Temperaturstabilität
stabil bis zu 40°C bei einer 15minütigen Behandlung bei pH 8,0,
(6) Substratspezifität
Phosphorsäure an der γ-PoBition von ATP oder dATP als Phosphatdonor wird auf Inosin oder Guanosin als Phosphatakzeptoren übertragen,
(7) Inhibitoren
Metallionen von Co^{2+}, Cu^{2+} und Zn^{2+},
(8) Aktivierung
das Enzym erfordert eine der beiden Ionengruppen: die eine ist K^+ und Mg^{2+} und die andere ist K^+ und Mn^{2+}.
(9) Km-Wert
Der Km-Wert in der Reaktionslösung. die zusammengesetzt ist aus 2 mM ATP, 10 mM $MgSO_4$, 300 mM KCl und 0.1 M HEPES-Puffer (pH 7,2) beträgt 2.1 mM für Inosin und 6.1 µM für Guanosin.
(10) Molekulargewicht
das Molekulargewicht. das von der Aminosäuresequenz abgeleitet ist. beträgt etwa 48 400 Daltons. eine Messung durch SDS-Polyacrylamidelektrophorese ergibt ein Molekulargewicht von etwa 43 000 Daltons.

2. Gereinigte Inosin-Guanosin-Kinase mit der folgenden Aminosäuresequenz:

```
  1  MetLysPheProGlyLysArgLysSerLysHisTyrPheProValAsnAlaArgAspPro
 21  LeuLeuGlnGlnPheGlnProGluAsnGluThrSerAlaAlaTrpValValGlyIleAsp
 41  GlnThrLeuValAspIleGluAlaLysValAspAspGluPheIleGluArgTyrGlyLeu
 61  SerAlaGlyHisSerLeuValIleGluAspAspValAlaGluAlaLeuTyrGlnGluLeu
 81  LysGlnLysAsnLeuIleThrHisGlnPheAlaGlyGlyThrIleGlyAsnThrMetHis
101  AsnTyrSerValLeuAlaAspAspArgSerValLeuLeuGlyValMetCysSerAsnIle
121  GluIleGlySerTyrAlaTyrArgTyrLeuCysAsnThrSerSerArgThrAspLeuAsn
141  TyrLeuGlnGlyValAspGlyProIleGlyArgCysPheThrLeuIleGlyGluSerGly
161  GluArgThrPheAlaIleSerProGlyHisMetAsnGlnLeuArgAlaGluSerIlePro
181  GluAspValIleAlaGlyAlaSerAlaLeuValLeuThrSerTyrLeuValArgCysLys
201  ProGlyGluProMetProGluAlaThrMetLysAlaIleGluTyrAlaLysLysTyrAsn
221  ValProValValLeuThrLeuGlyThrLysPheValIleAlaGluAsnProGlnTrpTrp
241  GlnGlnPheLeuLysAspHisValSerIleLeuAlaMetAsnGluAspGluAlaGluAla
261  LeuThrGlyGluSerAspProLeuLeuAlaSerAspLysAlaLeuAspTrpValAspLeu
281  ValLeuCysThrAlaGlyProIleGlyLeuTyrMetAlaGlyPheThrGluAspGluAla
301  LysArgLysThrGlnHisProLeuLeuProGlyAlaIleAlaGluPheAsnGlnTyrGlu
321  PheSerArgAlaMetArgHisLysAspCysGlnAsnProLeuArgValTyrSerHisIle
341  AlaProTyrMetGlyGlyProGluLysIleMetAsnThrAsnGlyAlaGlyAspGlyAla
361  LeuAlaAlaLeuLeuHisAspIleThrAlaAsnSerTyrHisArgSerAsnValProAsn
381  SerSerLysHisLysPheThrTrpLeuThrTyrSerSerLeuAlaGlnValCysLysTyr
401  AlaAsnArgValSerTyrGlnValLeuAsnGlnHisSerProArgLeuThrArgGlyLeu
421  ProGluArgGluAspSerLeuGluGluSerTyrTrpAspArg
```

3. Gen. das eine Inosin-Guanosin-Kinase mit der Aminosäuresequenz gemäß der Definition in Anspruch 2 codiert.

4. Gen nach Anspruch 3. wobei das Gen die folgende Nucleotidsequenz aufweist:

```
   1  ATGAAATTTC CGGGTAAACG TAAATCCAAA CATTACTTCC CCGTAAACGC
  51  ACGCGATCCG CTGCTTCAGC AATTCCAGCC AGAAAACGAA ACCAGCGCTG
 101  CCTGGGTAGT GGGTATCGAT CAAACGCTGG TCGATATTGA AGCGAAAGTG
 151  GATGATGAAT TTATTGAGCG TTATGGATTA AGCGCCGGGC ATTCACTGGT
 201  GATTGAGGAT GATGTAGCCG AAGCGCTTTA TCAGGAACTA AAACAGAAAA
 251  ACCTGATTAC CCATCAGTTT GCGGGTGGCA CCATTGGTAA CACCATGCAC
 301  AACTACTCGG TGCTCGCGGA CGACCGTTCG GTGCTGCTGG GCGTCATGTG
 351  CAGCAATATT GAAATTGGCA GTTATGCCTA TCGTTACCTG TGTAACACTT
 401  CCAGCCGTAC CGATCTTAAC TATCTACAAG GCGTGGATGG CCCGATTGGT
 451  CGTTGCTTTA CGCTGATTGG CGAGTCCGGG GAACGTACCT TTGCTATCAG
 501  TCCAGGCCAC ATGAACCAGC TGCGGGCTGA AAGCATTCCG GAAGATGTGA
 551  TTGCCGGAGC CTCGGCACTG GTTCTCACCT CATATCTGGT GCGTTGCAAG
 601  CCGGGTGAAC CCATGCCGGA AGCAACCATG AAAGCCATTG AGTACGCGAA
 651  GAAATATAAC GTACCGGTGG TGCTGACGCT GGGCACCAAG TTTGTCATTG
 701  CCGAGAATCC GCAGTGGTGG CAGCAATTCC TCAAAGATCA CGTCTCTATC
 751  CTTGCGATGA ACGAAGATGA AGCCGAAGCG TTGACCGGAG AAAGCGATCC
 801  GTTGTTGGCA TCTGACAAGG CGCTGGACTG GGTAGATCTG GTGCTGTGCA
 851  CCGCCGGGCC AATCGGCTTG TATATGGCGG GCTTTACCGA AGACGAAGCG
 901  AAACGTAAAA CCCAGCATCC GCTGCTGCCG GGCGCTATAG CGGAATTCAA
 951  CCAGTATGAG TTTAGCCGCG CCATGCGCCA CAAGGATTGC CAGAATCCGC
1001  TGCGTGTATA TTCGCACATT GCGCCGTACA TGGGCGGGCC GGAAAAAATC
1051  ATGAACACTA ATGGAGCGGG GGATGGCGCA TTGGCAGCGT TGCTGCATGA
1101  CATTACCGCC AACAGCTACC ATCGTAGCAA CGTACCAAAC TCCAGCAAAC
1151  ATAAATTCAC CTGGTTAACT TATTCATCGT TAGCGCAGGT GTGTAAATAT
1201  GCTAACCGTG TGAGCTATCA GGTACTGAAC CAGCATTCAC CTCGTTTAAC
1251  GCGCGGCTTG CCGGAGCGTG AAGACAGCCT GGAAGAGTCT TACTCGGATC
1301  GT
```

5. Gen nach Anspruch 3 oder 4, wobei das Gen von Escherichia coli stammt.

6. Rekombinante DNA, erhalten durch Insertion eines ein Gen gemäß der Definition in einem der Ansprüche 3 bis 5 enthaltenden DNA-Fragments in einen Vektor.

7. Mikroorganismus, der eine rekombinante DNA gemäß der Definition in Anspruch 6 trägt.

8. Mikroorganismus nach Anspruch 7, wobei der Mikroorganismus Escherichia coli ist.

9. Verfahren zur Herstellung einer Inosin-Guanosin-Kinase, umfassend die Züchtung eines Mikroorganismus, der zur Gattung Escherichia gehört und eine rekombinante DNA gemäß der Definition in Anspruch 6 trägt, in einem Medium zur Erzeugung und Anreicherung der Inosin-Guanosin-Kinase in den gezüchteten Zellen und die Gewinnung der Inosin-Guanosin-Kinase aus der Kultur.

10. Verfahren zur Herstellung von 5'-IMP oder 5'-GMP, umfassend die Umsetzung von Inosin oder Guanosin mit einem Phosphatdonor in einem wäßrigen Medium in Gegenwart einer Enzymquelle, die (1) eine gereinigte Inosin-Guanosin-Kinase gemäß der Definition in Anspruch 1 oder 2 oder (2) eine Zelle, eine Kulturbrühe oder ein nach Behandlung der Mikroorganismen gemäß der Definition in Anspruch 7 erhaltenes Produkt ist, zur Erzeugung und Anreicherung von 5'-IMP oder 5'-GMP im Reaktionsgemisch und Gewinnung von 5'-IMP oder 5'-GMP aus dem Reaktionsgemisch.

11. Verfahren zur Herstellung von 5'-IMP oder 5'-GMP nach Anspruch 10, wobei der Phosphatdonor ATP oder dATP ist.

12. Verfahren zur Herstellung von 5'-IMP oder 5'-GMP nach Anspruch 10, wobei der Mikroorganismus ein Mikroorganismus der Gattung Escherichia ist.

Patentansprüche für folgenden Vertragsstaat : ES

1. Verfahren zur Herstellung einer Inosin-Guanosin-Kinase, umfassend die Züchtung eines Mikroorganismus, der zur Gattung Escherichia gehört und eine rekombinante DNA trägt, die durch Insertion eines ein Gen, das eine Inosin-Guanosin-Kinase mit der folgenden Aminosäuresequenz codiert, enthaltenden DNA-Fragments in einen Vektor erhalten wurde, in einem Medium:

```
  1  MetLysPheProGlyLysArgLysSerLysHisTyrPheProValAsnAlaArgAspPro
 21  LeuLeuGlnGlnPheGlnProGluAsnGluThrSerAlaAlaTrpValValGlyIleAsp
 41  GlnThrLeuValAspIleGluAlaLysValAspAspGluPheIleGluArgTyrGlyLeu
 61  SerAlaGlyHisSerLeuValIleGluAspAspValAlaGluAlaLeuTyrGlnGluLeu
 81  LysGlnLysAsnLeuIleThrHisGlnPheAlaGlyGlyThrIleGlyAsnThrMetHis
101  AsnTyrSerValLeuAlaAspAspArgSerValLeuLeuGlyValMetCysSerAsnIle
121  GluIleGlySerTyrAlaTyrArgTyrLeuCysAsnThrSerSerArgThrAspLeuAsn
141  TyrLeuGlnGlyValAspGlyProIleGlyArgCysPheThrLeuIleGlyGluSerGly
161  GluArgThrPheAlaIleSerProGlyHisMetAsnGlnLeuArgAlaGluSerIlePro
181  GluAspValIleAlaGlyAlaSerAlaLeuValLeuThrSerTyrLeuValArgCysLys
201  ProGlyGluProMetProGluAlaThrMetLysAlaIleGluTyrAlaLysLysTyrAsn
221  ValProValValLeuThrLeuGlyThrLysPheValIleAlaGluAsnProGlnTrpTrp
241  GlnGlnPheLeuLysAspHisValSerIleLeuAlaMetAsnGluAspGluAlaGluAla
261  LeuThrGlyGluSerAspProLeuLeuAlaSerAspLysAlaLeuAspTrpValAspLeu
281  ValLeuCysThrAlaGlyProIleGlyLeuTyrMetAlaGlyPheThrGluAspGluAla
301  LysArgLysThrGlnHisProLeuLeuProGlyAlaIleAlaGluPheAsnGlnTyrGlu
321  PheSerArgAlaMetArgHisLysAspCysGlnAsnProLeuArgValTyrSerHisIle
341  AlaProTyrMetGlyGlyProGluLysIleMetAsnThrAsnGlyAlaGlyAspGlyAla
361  LeuAlaAlaLeuLeuHisAspIleThrAlaAsnSerTyrHisArgSerAsnValProAsn
381  SerSerLysHisLysPheThrTrpLeuThrTyrSerSerLeuAlaGlnValCysLysTyr
401  AlaAsnArgValSerTyrGlnValLeuAsnGlnHisSerProArgLeuThrArgGlyLeu
421  ProGluArgGluAspSerLeuGluGluSerTyrTrpAspArg
```

2. Verfahren nach Anspruch 1. dadurch gekennzeichnet. daß eine Inosin-Guanosin-Kinase mit den folgenden physikalisch-chemischen Eigenschaften hergestellt wird:

(1) Wirkung

Das Enzym katalysiert die Bildung von Adenosindiphosphat (ADP) und 5'-Inosinsäure (5'-IMP) aus Adenosintriphosphat (ATP) und Inosin. die Bildung von Desoxyadenosindiphosphat (dADP) und 5'-IMP aus Desoxyadenosintriphosphat (dATP) und Inosin. die Bildung von ADP und 5'-Guanylsäure (5'-GMP) aus ATP und Guanosin und die Bildung von dADP und 5'-GMP aus dATP und Guanosin.

(2) pH-Optimum

6.9 - 8.2.

(3) pH-Stabilität

das Enzym ist stabil in einem pH-Bereich von 6.6 bis 9.0 bei einer 16stündigen Behandlung bei 4°C. die Zugabe von KCl verbessert die Stabilität während der Lagerung.

(4) Temperaturoptimum
25-40°C,
(5) Temperaturstabilität
stabil bis zu 40°C bei einer 15minütigen Behandlung bei pH 8,0,
(6) Substratspezifität
Phosphorsäure an der γ-Position von ATP oder dATP als Phosphatdonor wird auf Inosin oder Guanosin als Phosphatakzeptoren übertragen,
(7) Inhibitoren
Metallionen von Co^{2+}, Cu^{2+} und Zn^{2+},
(8) Aktivierung
das Enzym erfordert eine der beiden Ionengruppen: die eine ist K^+ und Mg^{2+} und die andere ist K^+ und Mn^{2+},
(9) Km-Wert
Der Km-Wert in der Reaktionslösung, die zusammengesetzt ist aus 2 mM ATP, 10 mM $MgSO_4$, 300 mM KCl und 0,1 M HEPES-Puffer (pH 7,2) beträgt 2,1 mM für Inosin und 6,1 µM für Guanosin,
(10) Molekulargewicht
das Molekulargewicht, das von der Aminosäuresequenz abgeleitet ist, beträgt etwa 48 400 Daltons, eine Messung durch SDS-Polyacrylamidelektrophorese ergibt ein Molekulargewicht von etwa 43 000 Daltons.

3. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß das Gen, das in dem in den Vektor inserierten DNA-Fragment enthalten ist, die folgende Nucleotidsequenz aufweist:

```
   1  ATGAAATTTC CCGGTAAACG TAAATCCAAA CATTACTTCC CCGTAAACGC
  51  ACGCGATCCG CTGCTTCAGC AATTCCAGCC AGAAAACGAA ACCAGCGCTG
 101  CCTGGGTAGT GGGTATCGAT CAAACGCTGG TCGATATTGA AGCGAAAGTG
 151  GATGATGAAT TTATTGAGCG TTATGGATTA AGCGCCGGGC ATTCACTGGT
 201  GATTGAGGAT GATGTAGCCG AAGCGCTTTA TCAGGAACTA AAACAGAAAA
 251  ACCTGATTAC CCATCAGTTT GCGGGTGGCA CCATTGGTAA CACCATGCAC
 301  AACTACTCGG TGCTCGCGGA CGACCGTTCG GTGCTGCTGG GCGTCATGTG
 351  CAGCAATATT GAAATTGGCA GTTATGCCTA TCGTTACCTG TGTAACACTT
 401  CCAGCCGTAC CGATCTTAAC TATCTACAAG GCGTGGATGG CCCGATTGGT
 451  CGTTGCTTTA CGCTGATTGG CGAGTCCGGG GAACGTACCT TTGCTATCAG
 501  TCCAGGCCAC ATGAACCAGC TGCGGGCTGA AAGCATTCCG GAAGATGTGA
 551  TTGCCGGAGC CTCGGCACTG GTTCTCACCT CATATCTGGT GCGTTGCAAG
 601  CCGGGTGAAC CCATGCCGGA AGCAACCATG AAAGCCATTG AGTACGCGAA
 651  GAAATATAAC GTACCGGTGG TGCTGACGCT GGGCACCAAG TTTGTCATTG
 701  CCGAGAATCC GCAGTGGTGG CAGCAATTCC TCAAAGATCA CGTCTCTATC
 751  CTTGCGATGA ACGAAGATGA AGCCGAAGCG TTGACCGGAG AAAGCGATCC
 801  GTTGTTGGCA TCTGACAAGG CGCTGGACTG GCTAGATCTG GTGCTGTGCA
 851  CCGCCGGGCC AATCGGCTTG TATATGGCGG GCTTTACCGA AGACGAAGCG
 901  AAACGTAAAA CCCAGCATCC GCTGCTGCCG GGCGCTATAG CGGAATTCAA
 951  CCAGTATGAG TTTAGCCGCG CCATGCGCCA CAAGGATTGC CAGAATCCGC
1001  TGCGTGTATA TTCGCACATT GCGCCGTACA TGGCCGGGCC GGAAAAAATC
1051  ATGAACACTA ATGGAGCGGG GGATGCCGCA TTGGCAGCGT TGCTGCATGA
1101  CATTACCGCC AACAGCTACC ATCGTAGCAA CGTACCAAAC TCCAGCAAAC
1151  ATAAATTCAC CTGGTTAACT TATTCATCGT TAGCGCAGGT GTGTAAATAT
1201  GCTAACCGTG TGAGCTATCA GGTACTGAAC CAGCATTCAC CTCGTTTAAC
1251  GCGCGGCTTG CCGGAGCGTG AAGACAGCCT GGAAGAGTCT TACTGGGATC
1301  GT
```

4. Verfahren zur Herstellung eines eine Inosin-Guanosin-Kinase codierenden Gens, umfassend die Clonierung und Isolierung eines Gens, das eine Inosin-Guanosin-Kinase mit der Aminosäuresequenz gemäß der Definition in Anspruch 1 codiert.

5. Verfahren nach Anspruch 4, dadurch gekennzeichnet, daß das clonierte und isolierte Gen durch die folgende Nucleotidsequenz gezeigt wird:

```
   1  ATGAAATTTC CCGGTAAACG TAAATCCAAA CATTACTTCC CCGTAAACGC
  51  ACGCGATCCG CTGCTTCAGC AATTCCAGCC AGAAAACGAA ACCAGCGCTG
 101  CCTGGGTAGT GGGTATCGAT CAAACGCTGG TCGATATTGA AGCGAAAGTG
 151  GATGATGAAT TTATTGAGCG TTATGGATTA AGCGCCGGGC ATTCACTGGT
 201  GATTGAGGAT GATGTAGCCG AAGCGCTTTA TCAGGAACTA AAACAGAAAA
 251  ACCTGATTAC CCATCAGTTT GCGGGTGGCA CCATTGGTAA CACCATGCAC
 301  AACTACTCGG TGCTCCGCGA CGACCGTTCG GTGCTGCTGG GCGTCATGTG
 351  CAGCAATATT GAAATTGGCA GTTATGCCTA TCGTTACCTG TGTAACACTT
 401  CCAGCCGTAC CGATCTTAAC TATCTACAAG GCGTGGATGG CCCGATTGGT
 451  CGTTGCTTTA CGCTGATTGG CGAGTCCGGG GAACGTACCT TTGCTATCAG
 501  TCCAGGCCAC ATGAACCAGC TGCGGGCTGA AAGCATTCCG GAAGATGTGA
 551  TTGCCGGAGC CTCGGCACTG GTTCTCACCT CATATCTGGT GCGTTGCAAG
 601  CCGGGTGAAC CCATGCCCGA AGCAACCATG AAAGCCATTG AGTACGCGAA
 651  GAAATATAAC GTACCGGTGG TGCTGACGCT GGGCACCAAG TTTGTCATTG
 701  CCGAGAATCC GCAGTGGTGG CAGCAATTCC TCAAAGATCA CGTCTCTATC
 751  CTTGCGATGA ACGAAGATGA AGCCGAAGCG TTGACCGGAG AAAGCGATCC
 801  GTTGTTGGCA TCTGACAAGG CGCTGGACTG GGTAGATCTG GTGCTGTGCA
 851  CCGCCGGGCC AATCGGCTTG TATATGGCGG GCTTTACCGA AGACGAAGCG
 901  AAACGTAAAA CCCAGCATCC GCTGCTGCCG GGCGCTATAG CGGAATTCAA
 951  CCAGTATGAG TTTAGCCGCG CCATGCGCCA CAAGGATTGC CAGAATCCGC
1001  TGCGTGTATA TTCGCACATT GCGCCGTACA TGGGCGGGCC GGAAAAAATC
1051  ATGAACACTA ATGGAGCGGG GGATGGCGCA TTGGCAGCGT TGCTGCATGA
1101  CATTACCGCC AACAGCTACC ATCGTAGCAA CGTACCAAAC TCCAGCAAAC
1151  ATAAATTCAC CTGGTTAACT TATTCATCGT TAGCGCAGGT GTGTAAATAT
1201  GCTAACCGTG TGAGCTATCA GGTACTGAAC CAGCATTCAC CTCGTTTAAC
1251  GCGCGGCTTG CCGGAGCGTG AAGACAGCCT GGAAGAGTCT TACTGGGATC
1301  GT
```

6. Verfahren nach Anspruch 4 oder 5, wobei das Gen von Escherichia coli stammt.

7. Verfahren zur Herstellung einer rekombinanten DNA, umfassend die Insertion eines DNA-Fragments, das ein nach einem der Verfahren nach den Ansprüchen 4 bis 6 hergestelltes Gen enthält, in einen Vektor.

8. Verfahren zur Herstellung von 5'-IMP oder 5'-GMP, umfassend die Umsetzung von Inosin oder Guanosin mit einem Phosphatdonor in einem wäßrigen Medium in Gegenwart einer Enzymquelle, die (1) eine Inosin-Guanosin-Kinase gemäß der Definition in Anspruch 1 oder (2) eine Zelle, eine Kulturbrühe oder ein nach Behandlung der Mikroorganismen, die eine rekombinante DNA gemäß der Definition in Anspruch 7 tragen, erhaltenes Produkt ist, zur Erzeugung und Anreicherung von 5'-IMP oder 5'-GMP im Reaktionsgemisch und Gewinnung von 5'-IMP oder 5'-GMP aus dem Reaktionsgemisch.

9. Verfahren zur Herstellung von 5'-IMP oder 5'-GMP nach Anspruch 8, wobei der Phosphatdonor ATP oder dATP ist.

10. Verfahren zur Herstellung von 5'-IMP oder 5'-GMP nach Anspruch 8, wobei der Mikroorganismus ein Mikroorganismus der Gattung Escherichia ist.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, LI, DE, FR, GB, IT, LU, NL, SE

1. Inosine-guanosine kinase purifiée, présentant les propriétés physico-chimiques suivantes :

a) action : cette enzyme exerce une action de formation d'adénosine-diphosphate (ADP) et d'acide 5'-inosinique (5'-IMP) à partir d'adénosine-triphosphate (ATP) et d'inosine, une action de formation de désoxyadénosine-diphosphate (dADP) et de 5'-IMP à partir de désoxyadénosine-triphosphate (dATP) et d'inosine, une action de formation d'ADP et d'acide 5'-guanylique (5'-GMP) à partir d'ATP et de guanosine, et une action de formation de dADP et de 5'-GMP à partir de dATP et de guanosine ;
b) pH optimal : 6,9-8,2 ;
c) stabilité vis-à-vis du pH : cette enzyme est stable dans le domaine de pH allant de 6,6 à 9,0, lors d'un traitement à 4°C pendant 16 heures ; l'addition de KCl améliore la stabilité en cours de conservation ;
d) température optimale : 25-40°C ;
e) stabilité vis-à-vis de la température : cette enzyme est stable jusqu'à 40°C, lors d'un traitement de 15 minutes à pH 8,0 ;
f) spécificité pour le substrat : le résidu d'acide phosphorique situé en position γ dans l'ATP ou le dATP, utilisés comme donneurs de phosphate, est transféré sur l'inosine ou la guanosine, utilisées comme accepteurs de phosphate ;
g) inhibiteurs : ions métalliques Co^{2+}, Cu^{2+} et zn^{2+} ;
h) activation : cette enzyme a besoin de l'une des deux paires d'ions suivantes : (K^+, Mg^{2+}) et (K^+, Mn^{2+}) ;
i) valeur de K_m : dans un mélange réactionnel contenant 2 mM d'ATP, 10 mM de $MgSO_4$, 300 mM de KCl et 0,1 M de tampon HEPES (pH 7,2), K_m vaut 2,1 mM dans le cas de l'inosine, et 6,1 µM dans le cas de la guanosine ;
j) masse moléculaire : la masse moléculaire déduite de la séquence d'acides aminés vaut environ 48400 daltons. et d'après les mesures effectuées par électrophorèse en gel de polyacrylamide-SDS. la masse moléculaire vaut environ 43000 daltons.

2. Inosine-guanosine kinase purifiée, représentée par la séquence suivante de résidus d'acides aminés :

```
  1  MetLysPheProGlyLysArgLysSerLysHisTyrPheProValAsnAlaArgAspPro
 21  LeuLeuGlnGlnPheGlnProGluAsnGluThrSerAlaAlaTrpValValGlyIleAsp
 41  GlnThrLeuValAspIleGluAlaLysValAspAspGluPheIleGluArgTyrGlyLeu
 61  SerAlaGlyHisSerLeuValIleGluAspAspValAlaGluAlaLeuTyrGlnGluLeu
 81  LysGlnLysAsnLeuIleThrHisGlnPheAlaGlyGlyThrIleGlyAsnThrMetHis
101  AsnTyrSerValLeuAlaAspAspArgSerValLeuLeuGlyValMetCysSerAsnIle
121  GluIleGlySerTyrAlaTyrArgTyrLeuCysAsnThrSerSerArgThrAspLeuAsn
141  TyrLeuGlnGlyValAspGlyProIleGlyArgCysPheThrLeuIleGlyGluSerGly
161  GluArgThrPheAlaIleSerProGlyHisMetAsnGlnLeuArgAlaGluSerIlePro
181  GluAspValIleAlaGlyAlaSerAlaLeuValLeuThrSerTyrLeuValArgCysLys
201  ProGlyGluProMetProGluAlaThrMetLysAlaIleGluTyrAlaLysLysTyrAsn
221  ValProValValLeuThrLeuGlyThrLysPheValIleAlaGluAsnProGlnTrpTrp
241  GlnGlnPheLeuLysAspHisValSerIleLeuAlaMetAsnGluAspGluAlaGluAla
261  LeuThrGlyGluSerAspProLeuLeuAlaSerAspLysAlaLeuAspTrpValAspLeu
281  ValLeuCysThrAlaGlyProIleGlyLeuTyrMetAlaGlyPheThrGluAspGluAla
301  LysArgLysThrGlnHisProLeuLeuProGlyAlaIleAlaGluPheAsnGlnTyrGlu
321  PheSerArgAlaMetArgHisLysAspCysGlnAsnProLeuArgValTyrSerHisIle
341  AlaProTyrMetGlyGlyProGluLysIleMetAsnThrAsnGlyAlaGlyAspGlyAla
361  LeuAlaAlaLeuLeuHisAspIleThrAlaAsnSerTyrHisArgSerAsnValProAsn
381  SerSerLysHisLysPheThrTrpLeuThrTyrSerSerLeuAlaGlnValCysLysTyr
401  AlaAsnArgValSerTyrGlnValLeuAsnGlnHisSerProArgLeuThrArgGlyLeu
421  ProGluArgGluAspSerLeuGluGluSerTyrTrpAspArg
```

3. Gène codant une inosine-guanosine kinase présentant la séquence de résidus d'acides aminés indiquée dans la revendication 2.

4. Gène conforme à la revendication 3. qui présente la séquence suivante de nucléotides :

```
   1  ATGAAATTTC CCGGTAAACG TAAATCCAAA CATTACTTCC CCGTAAACGC
  51  ACGCGATCCG CTGCTTCAGC AATTCCAGCC AGAAAACGAA ACCAGCGCTG
 101  CCTGGGTAGT GGGTATCGAT CAAACGCTGG TCGATATTGA AGCGAAAGTG
 151  GATGATGAAT TTATTGAGCG TTATGGATTA AGCGCCGGGC ATTCACTGGT
 201  GATTGAGGAT GATGTAGCCG AAGCGCTTTA TCAGGAACTA AAACAGAAAA
 251  ACCTGATTAC CCATCAGTTT GCGGGTGGCA CCATTGGTAA CACCATGCAC
 301  AACTACTCGG TGCTCGCGGA CGACCGTTCG GTGCTGCTGG GCGTCATGTG
 351  CAGCAATATT GAAATTGGCA GTTATGCCTA TCGTTACCTG TGTAACACTT
 401  CCAGCCGTAC CGATCTTAAC TATCTACAAG GCGTGGATGG CCCGATTGGT
 451  CGTTGCTTTA CGCTGATTGG CGAGTCCGGG GAACGTACCT TTGCTATCAG
 501  TCCAGGCCAC ATGAACCAGC TGCGGGCTGA AAGCATTCCG GAAGATGTGA
 551  TTGCCGGAGC CTCGGCACTG GTTCTCACCT CATATCTGGT GCGTTGCAAG
 601  CCGGGTGAAC CCATGCCGGA AGCAACCATG AAAGCCATTG AGTACGCGAA
 651  GAAATATAAC GTACCGGTGG TGCTGACGCT GGGCACCAAG TTTGTCATTG
 701  CCGAGAATCC GCAGTGGTGG CAGCAATTCC TCAAAGATCA CGTCTCTATC
 751  CTTGCGATGA ACGAAGATGA AGCCGAAGCG TTGACCGGAG AAAGCGATCC
 801  GTTGTTGGCA TCTGACAAGG CGCTGGACTG GGTAGATCTG GTGCTGTGCA
 851  CCGCCGGGCC AATCGGCTTG TATATGGCGG GCTTTACCGA AGACGAAGCG
 901  AAACGTAAAA CCCAGCATCC GCTGCTGCCG GGCGCTATAG CGGAATTCAA
 951  CCAGTATGAG TTTAGCCGCG CCATGCGCCA CAAGGATTGC CAGAATCCGC
1001  TGCGTGTATA TTCGCACATT GCGCCGTACA TGGGCGGGCC GGAAAAAATC
1051  ATGAACACTA ATGGAGCGGG GGATGGCGCA TTGGCAGCGT TGCTGCATGA
1101  CATTACCGCC AACAGCTACC ATCGTAGCAA CGTACCAAAC TCCAGCAAAC
1151  ATAAATTCAC CTGGTTAACT TATTCATCGT TAGCGCAGGT GTGTAAATAT
1201  GCTAACCGTG TGAGCTATCA GGTACTGAAC CAGCATTCAC CTCGTTTAAC
1251  GCGCGGCTTG CCGGAGCGTG AAGACAGCCT GGAAGAGTCT TACTGGGATC
1301  GT
```

5. Gène conforme à la revendication 3 ou 4, qui provient d'Escherichia coli.

6. ADN recombiné obtenu par insertion, dans un vecteur, d'un fragment d'ADN contenant un gène conforme à l'une des revendications 3 à 5.

7. Microorganisme hébergeant un ADN recombiné conforme à la revendication 6.

8. Microorganisme conforme à la revendication 7, appartenant à l'espèce Escherichia coli.

9. Procédé de préparation d'une inosine-guanosine kinase, lequel procédé comporte le fait de cultiver, dans un milieu, un microorganisme appartenant au genre Escherichia et hébergeant un ADN recombiné conforme à la revendication 6, de façon à produire et laisser s'accumuler de l'inosine-guanosine kinase dans les cellules cultivées, et le fait de récupérer l'inosine-guanosine kinase dans la culture.

10. Procédé de préparation de 5'-IMP ou de 5'-GMP, lequel procédé comporte le fait de faire réagir de l'inosine ou de la guanosine avec un donneur de phosphate, dans un milieu aqueux et en présence d'une source d'enzyme qui est (1) une inosine-guanosine kinase purifiée conforme à la revendication 1 ou 2, ou (2) des cellules, un bouillon de culture ou un produit de traitement d'un microorganisme conforme à la revendication 7, de façon à produire et laisser s'accumuler du 5'-IMP ou du 5'-GMP dans le mélange réactionnel, et le fait de récupérer le 5'-IMP ou le 5'-GMP dans le mélange réactionnel.

11. Procédé de préparation de 5'-IMP ou de 5'-GMP, conforme à la revendication 10, dans lequel ledit donneur de phosphate est de l'ATP ou du dATP.

12. Procédé de préparation de 5'-IMP ou de 5'-GMP, conforme à la revendication 10, dans lequel ledit microorganisme est un microorganisme appartenant au genre Escherichia.

Revendications pour l'Etat Contractant suivant : ES

1. Procédé de préparation d'une inosine-guanosine kinase, lequel procédé comporte le fait de cultiver, dans un milieu, un microorganisme appartenant au genre Escherichia et hébergeant un ADN recombiné obtenu par insertion, dans un vecteur, d'un fragment d'ADN qui contient un gène codant une inosine-guanosine kinase présentant la séquence suivante de résidus d'acides aminés :

```
  1  MetLysPheProGlyLysArgLysSerLysHisTyrPheProValAsnAlaArgAspPro
 21  LeuLeuGlnGlnPheGlnProGluAsnGluThrSerAlaAlaTrpValValGlyIleAsp
 41  GlnThrLeuValAspIleGluAlaLysValAspAspGluPheIleGluArgTyrGlyLeu
 61  SerAlaGlyHisSerLeuValIleGluAspAspValAlaGluAlaLeuTyrGlnGluLeu
 81  LysGlnLysAsnLeuIleThrHisGlnPheAlaGlyGlyThrIleGlyAsnThrMetHis
101  AsnTyrSerValLeuAlaAspAspArgSerValLeuLeuGlyValMetCysSerAsnIle
121  GluIleGlySerTyrAlaTyrArgTyrLeuCysAsnThrSerSerArgThrAspLeuAsn
141  TyrLeuGlnGlyValAspGlyProIleGlyArgCysPheThrLeuIleGlyGluSerGly
161  GluArgThrPheAlaIleSerProGlyHisMetAsnGlnLeuArgAlaGluSerIlePro
181  GluAspValIleAlaGlyAlaSerAlaLeuValLeuThrSerTyrLeuValArgCysLys
201  ProGlyGluProMetProGluAlaThrMetLysAlaIleGluTyrAlaLysLysTyrAsn
221  ValProValValLeuThrLeuGlyThrLysPheValIleAlaGluAsnProGlnTrpTrp
241  GlnGlnPheLeuLysAspHisValSerIleLeuAlaMetAsnGluAspGluAlaGluAla
261  LeuThrGlyGluSerAspProLeuLeuAlaSerAspLysAlaLeuAspTrpValAspLeu
281  ValLeuCysThrAlaGlyProIleGlyLeuTyrMetAlaGlyPheThrGluAspGluAla
301  LysArgLysThrGlnHisProLeuLeuProGlyAlaIleAlaGluPheAsnGlnTyrGlu
321  PheSerArgAlaMetArgHisLysAspCysGlnAsnProLeuArgValTyrSerHisIle
341  AlaProTyrMetGlyGlyProGluLysIleMetAsnThrAsnGlyAlaGlyAspGlyAla
361  LeuAlaAlaLeuLeuHisAspIleThrAlaAsnSerTyrHisArgSerAsnValProAsn
381  SerSerLysHisLysPheThrTrpLeuThrTyrSerSerLeuAlaGlnValCysLysTyr
401  AlaAsnArgValSerTyrGlnValLeuAsnGlnHisSerProArgLeuThrArgGlyLeu
421  ProGluArgGluAspSerLeuGluGluSerTyrTrpAspArg
```

2. Procédé conforme à la revendication 1, caractérisé en ce que l'on prépare une inosine-guanosine kinase purifiée qui présente les propriétés physico-chimiques suivantes :

a) action : cette enzyme exerce une action de formation d'adénosine-diphosphate (ADP) et d'acide 5'-inosinique (5'-IMP) à partir d'adénosine-triphosphate (ATP) et d'inosine, une action de formation de désoxyadénosine-diphosphate (dADP) et de 5'-IMP à partir de désoxyadénosine-triphosphate (dATP) et d'inosine, une action de formation d'ADP et d'acide 5'-guanylique (5'-GMP) à partir d'ATP et de guanosine, et une action de formation de dADP et de 5'-GMP à partir de dATP et de guanosine :
b) pH optimal : 6.9-8.2 :
c) stabilité vis-à-vis du pH: cette enzyme est stable dans le domaine de pH allant de 6.6 à 9.0, lors d'un traitement à 4°C pendant 16 heures l'addition de KCl améliore la stabilité en cours de conservation :
d) température optimale : 25-40°C ;
e) stabilité vis-à-vis de la température: cette enzyme est stable jusqu'à 40°C, lors d'un traitement de 15 minutes à pH 8,0 :
f) spécificité pour le substrat: le résidu d'acide phosphorique situé en position γ dans l'ATP ou le dATP, utilisés

comme donneurs de phosphate, est transféré sur l'inosine ou la guanosine, utilisées comme accepteurs de phosphate ;
g) inhibiteurs: ions métalliques Co^{2+}, Cu^{2+} et Zn^{2+} ;
h) activation : cette enzyme a besoin de l'une des deux paires d'ions suivantes : (K^+, Mg^{2+}) et (K^+, Mn^{2+}) ;
i) valeur de K_m : dans un mélange réactionnel contenant 2 mM d'ATP, 10 mM de $MgSO_4$, 300 mM de KCl et 0,1 M de tampon HEPES (pH 7,2), K_m vaut 2,1 mM dans le cas de l'inosine, et 6,1 µM dans le cas de la guanosine ;
j) masse moléculaire : la masse moléculaire déduite de la séquence d'acides aminés vaut environ 48400 daltons, et d'après les mesures effectuées par électrophorèse en gel de polyacrylamide-SDS, la masse moléculaire vaut environ 43000 daltons.

3. Procédé conforme à la revendication 1, caractérisé en ce que le gène contenu dans le fragment d'ADN inséré dans le vecteur présente la séquence suivante de nucléotides :

```
   1  ATGAAATTTC CCGGTAAACG TAAATCCAAA CATTACTTCC CCGTAAACGC
  51  ACGCGATCCG CTGCTTCAGC AATTCCAGCC AGAAAACGAA ACCAGCGCTG
 101  CCTGGGTAGT GGGTATCGAT CAAACGCTGG TCGATATTGA AGCGAAAGTG
 151  GATGATGAAT TTATTGAGCG TTATGGATTA AGCGCCGGGC ATTCACTGGT
 201  GATTGAGGAT GATGTAGCCG AAGCGCTTTA TCAGGAACTA AAACAGAAAA
 251  ACCTGATTAC CCATCAGTTT GCGGGTGGCA CCATTGGTAA CACCATGCAC
 301  AACTACTCGG TGCTCGCGGA CGACCGTTCG GTGCTGCTGG GCGTCATGTG
 351  CAGCAATATT GAAATTGGCA GTTATGCCTA TCGTTACCTG TGTAACACTT
 401  CCAGCCGTAC CGATCTTAAC TATCTACAAG GCGTGGATGG CCCGATTGGT
 451  CGTTGCTTTA CGCTGATTGG CGAGTCCGGG GAACGTACCT TTGGTATCAG
 501  TCCAGGCCAC ATGAACCAGC TGCGGGCTGA AAGCATTCCG GAAGATGTGA
 551  TTGCCGGAGC CTCGGCACTG GTTCTCACCT CATATCTGGT GCGTTGCAAG
 601  CCGGGTGAAC CCATGCCGGA AGCAACCATG AAAGCCATTG AGTACGCGAA
 651  GAAATATAAC GTACCGGTGG TGCTGACGCT GGGCACCAAG TTTGTCATTG
 701  CCGAGAATCC GCAGTGGTGG CAGCAATTCC TCAAAGATCA CGTCTCTATC
 751  CTTGCGATGA ACGAAGATGA AGCCGAAGCG TTGACCGGAG AAAGCGATCC
 801  GTTGTTGGCA TCTGACAAGG CGCTGGACTG GGTAGATCTG GTGCTGTGCA
 851  CCGCCGGGCC AATCGGCTTG TATATGGCGG GCTTTACCGA AGACGAAGCG
 901  AAACGTAAAA CCCAGCATCC GCTGCTGCCG GGCGCTATAG CGGAATTCAA
 951  CCAGTATGAG TTTAGCCGCG CCATGCGCCA CAAGGATTGC CAGAATCCGC
1001  TGCGTGTATA TTCGCACATT GCGCCGTACA TGGGCGGGCC GGAAAAAATC
1051  ATGAACACTA ATGGAGCGGG GGATGGCGCA TTGGCAGCGT TGCTGCATGA
1101  CATTACCGCC AACAGCTACC ATCGTAGCAA CGTACCAAAC TCCAGCAAAC
1151  ATAAATTCAC CTGGTTAACT TATTCATCGT TAGCGCAGGT GTGTAAATAT
1201  GCTAACCGTG TGAGCTATCA GGTACTGAAC CAGCATTCAC CTCGTTTAAC
1251  GCGCGGCTTG CCGGAGCGTG AAGACAGCCT GGAAGAGTCT TACTGGGATC
1301  GT
```

4. Procédé de préparation d'un gène codant une inosine-guanosine kinase, lequel procédé comporte les étapes consistant à cloner et à isoler un gène codant une inosine-guanosine kinase présentant la séquence de résidus d'acides aminés indiquée dans la revendication 1.

5. Procédé conforme à la revendication 4, caractérisé en ce que le gène cloné et isolé est représenté par la séquence

suivante de nucléotides :

```
   1  ATGAAATTTC CCGGTAAACG TAAATCCAAA CATTACTTCC CCGTAAACGC
  51  ACGCGATCCG CTGCTTCAGC AATTCCAGCC AGAAAACGAA ACCAGCGCTG
 101  CCTGGGTAGT GGGTATCGAT CAAACGCTGG TCGATATTGA AGCGAAAGTG
 151  GATGATGAAT TTATTGAGCG TTATGGATTA AGCGCCGGGC ATTCACTGGT
 201  GATTGAGGAT GATGTAGCCG AAGCGCTTTA TCAGGAACTA AAACAGAAAA
 251  ACCTGATTAC CCATCAGTTT GCGGGTGGCA CCATTGGTAA CACCATGCAC
 301  AACTACTCGG TGCTCGCGGA CGACCGTTCG GTGCTGCTGG GCGTCATGTG
 351  CAGCAATATT GAAATTGGCA GTTATGCCTA TCGTTACCTG TGTAACACTT
 401  CCAGCCGTAC CGATCTTAAC TATCTACAAG GCGTGGATGG CCCGATTGGT
 451  CGTTGCTTTA CGCTGATTGG CGAGTCCGGG GAACGTACCT TTGCTATCAG
 501  TCCAGGCCAC ATGAACCAGC TGCGGGCTGA AAGCATTCCG GAAGATGTGA
 551  TTGCCGGAGC CTCGGCACTG GTTCTCACCT CATATCTGGT GCGTTGCAAG
 601  CCGGGTGAAC CCATGCCGGA AGCAACCATG AAAGCCATTG AGTACGCGAA
 651  GAAATATAAC GTACCGGTGG TGCTGACGCT GGGCACCAAG TTTGTCATTG
 701  CCGAGAATCC GCAGTGGTGG CAGCAATTCC TCAAAGATCA CGTCTCTATC
 751  CTTGCGATGA ACGAAGATGA AGCCGAAGCG TTGACCGGAG AAAGCGATCC
 801  GTTGTTGGCA TCTGACAAGG CGCTGGACTG GGTAGATCTG GTGCTGTGCA
 851  CCGCCGGGCC AATCGGCTTG TATATGGCGG GCTTTACCGA AGACGAAGCG
 901  AAACGTAAAA CCCAGCATCC GCTGCTGCCG GGCGCTATAG CGGAATTCAA
 951  CCAGTATGAG TTTAGCCGCG CCATGCGCCA CAAGGATTGC CAGAATCCGC
1001  TGCGTGTATA TTCGCACATT GCGCCGTACA TGGGCGGGCC GGAAAAAATC
1051  ATGAACACTA ATGGAGCGGG GGATGGCGCA TTGGCAGCGT TGCTGCATGA
1101  CATTACCGCC AACAGCTACC ATCGTAGCAA CGTACCAAAC TCCAGCAAAC
1151  ATAAATTCAC CTGGTTAACT TATTCATCGT TAGCGCAGGT GTGTAAATAT
1201  GCTAACCGTG TGAGCTATCA GGTACTGAAC CAGCATTCAC CTCGTTTAAC
1251  GCGCGGCTTG CCGGAGCGTG AAGACAGCCT GGAAGAGTCT TACTGGGATC
1301  GT
```

6. Procédé conforme à la revendication 4 ou 5, dans lequel ledit gène dérive d'Escherichia coli.

7. Procédé de production d'un ADN recombiné, qui comporte les étapes consistant à insérer, dans un vecteur, un fragment d'ADN contenant un gène préparé conformément à l'une des revendications 4 à 6.

8. Procédé de préparation de 5'-IMP ou de 5'-GMP, lequel procédé comporte le fait de faire réagir de l'inosine ou de la guanosine avec un donneur de phosphate, dans un milieu aqueux et en présence d'une source d'enzyme qui est (1) une inosine-guanosine kinase définie dans la revendication 1, ou (2) des cellules, un bouillon de culture ou un produit de traitement d'un microorganisme hébergeant un ADN recombiné préparé conformément à la revendication 7, de façon à produire et laisser s'accumuler du 5'-IMP ou du 5'-GMP dans le mélange réactionnel, et le fait de récupérer le 5'-IMP ou le 5'-GMP dans le mélange réactionnel.

9. Procédé de préparation de 5'-IMP ou de 5'-GMP, conforme à la revendication 8, dans lequel ledit donneur de phosphate est de l'ATP ou du dATP.

10. Procédé de préparation de 5'-IMP ou de 5'-GMP, conforme à la revendication 8, dans lequel ledit microorganisme est un microorganisme appartenant au genre Escherichia.

FIG.1

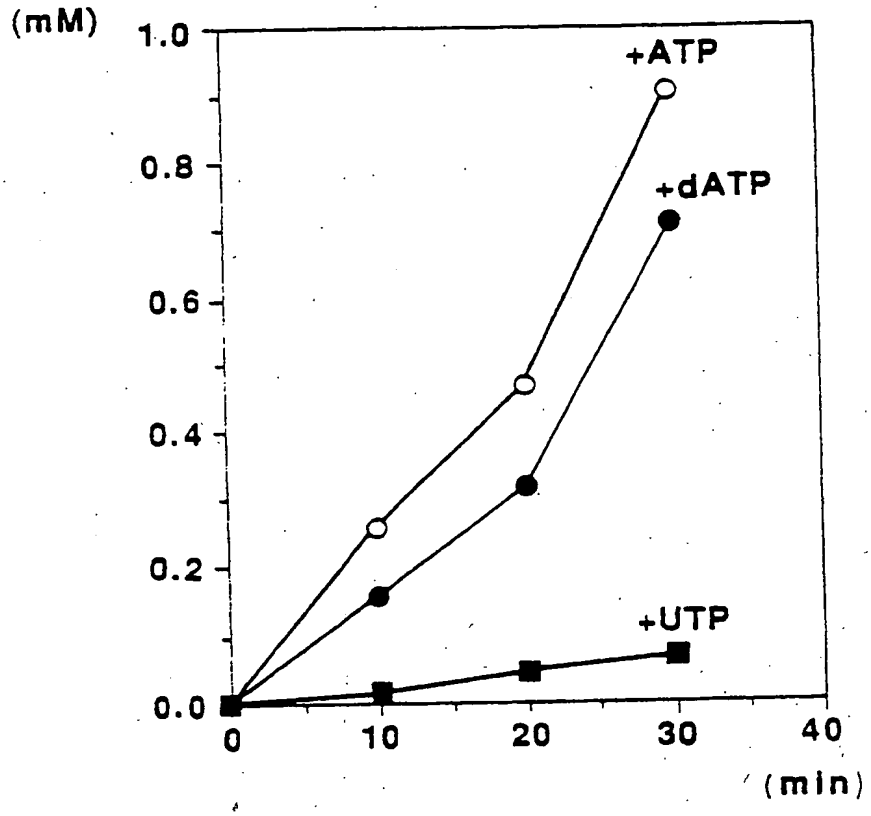


FIG.2

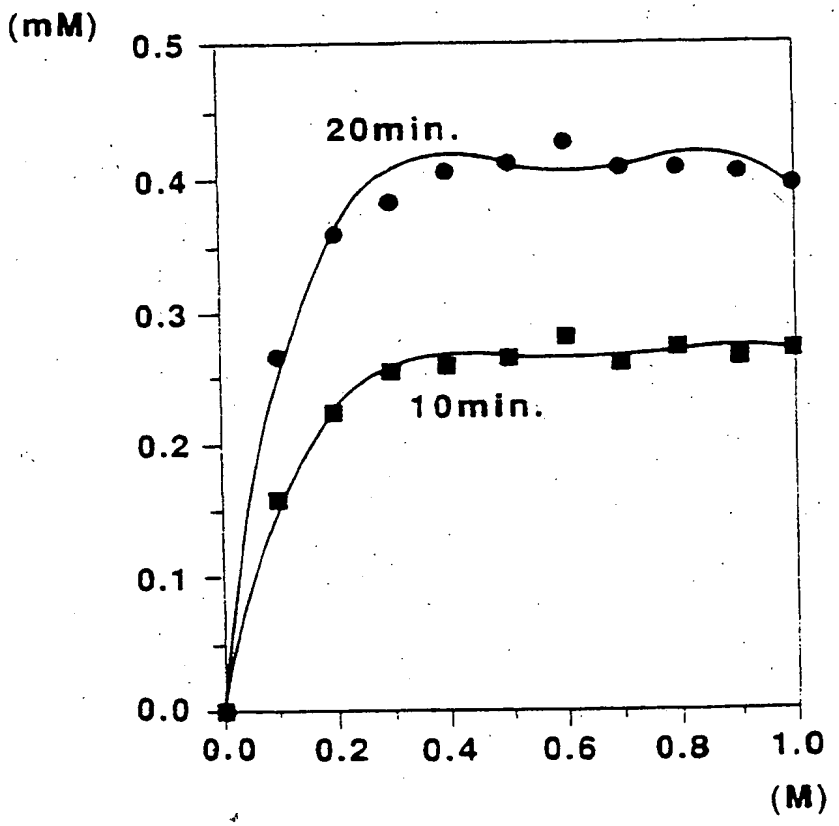


FIG. 3

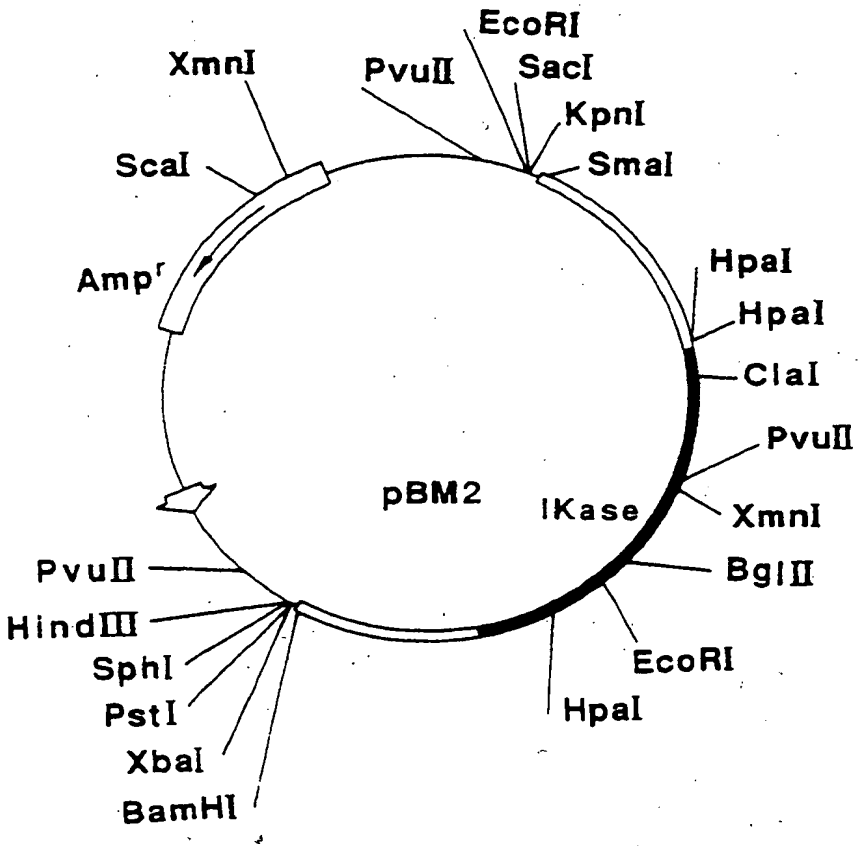

EcoRI
XmnI
PvuII
SacI
KpnI
ScaI
SmaI
Amp^r
HpaI
HpaI
ClaI
PvuII
pBM2
IKase
XmnI
BglII
PvuII
HindIII
SphI
EcoRI
PstI
HpaI
XbaI
BamHI

FIG. 4

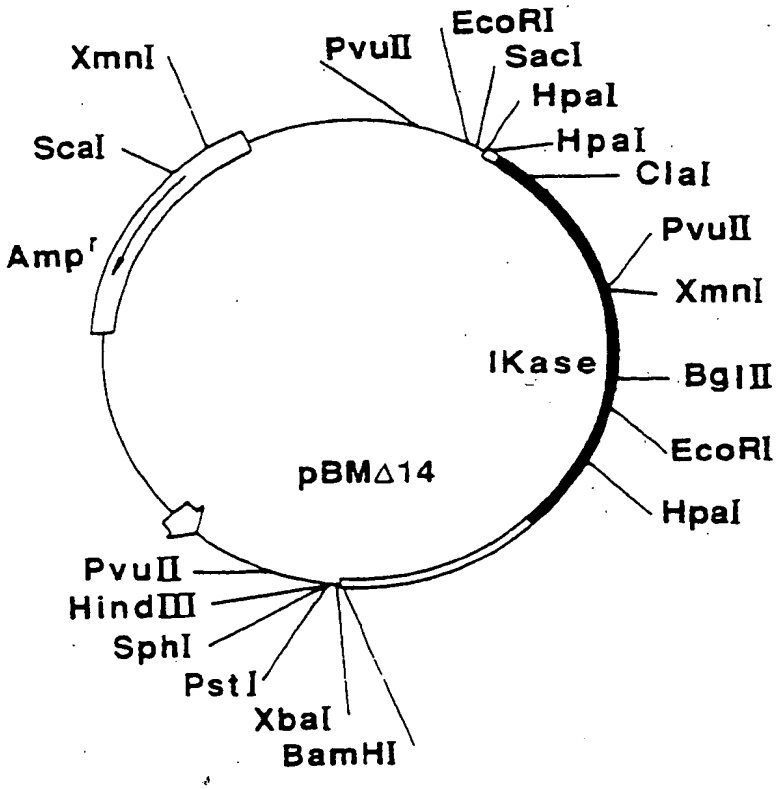

EcoRI
PvuII
SacI
XmnI
HpaI
ScaI
HpaI
ClaI
PvuII
Amp^r
XmnI
IKase
BglII
EcoRI
pBMΔ14
HpaI
PvuII
HindIII
SphI
PstI
XbaI
BamHI

FIG. 5

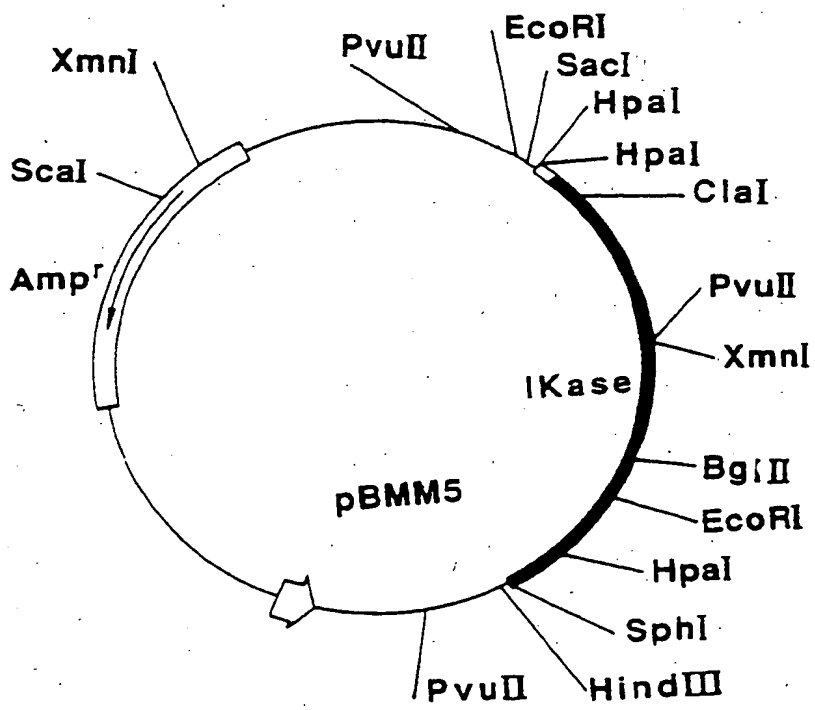

XmnI
PvuII
EcoRI
SacI
HpaI
HpaI
ClaI
ScaI
Ampr
PvuII
XmnI
IKase
BglII
pBMM5
EcoRI
HpaI
SphI
PvuII
HindIII

FIG.6

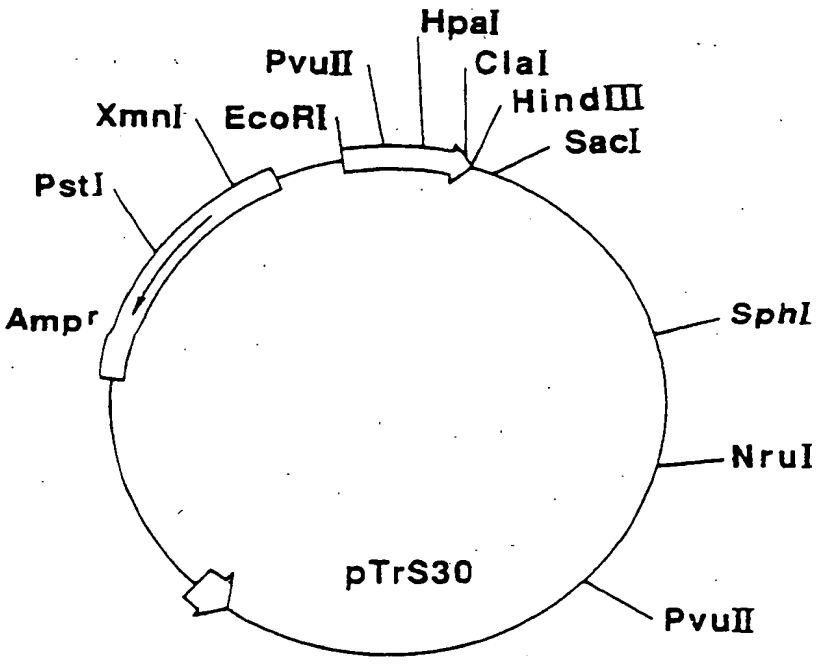

HpaI
PvuII
ClaI
HindIII
XmnI
EcoRI
SacI
PstI
Ampr
SphI
NruI
pTrS30
PvuII

FIG. 7

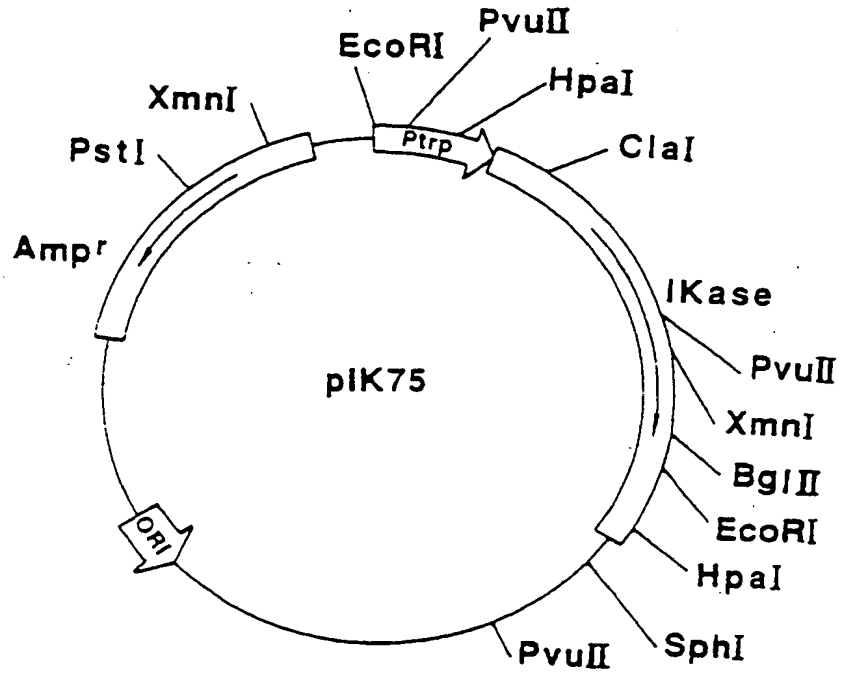

PvuII
EcoRI
HpaI
XmnI
PstI
Ptrp
ClaI
Ampr
IKase
pIK75
PvuII
XmnI
BglII
EcoRI
HpaI
ORI
PvuII
SphI

FIG. 8

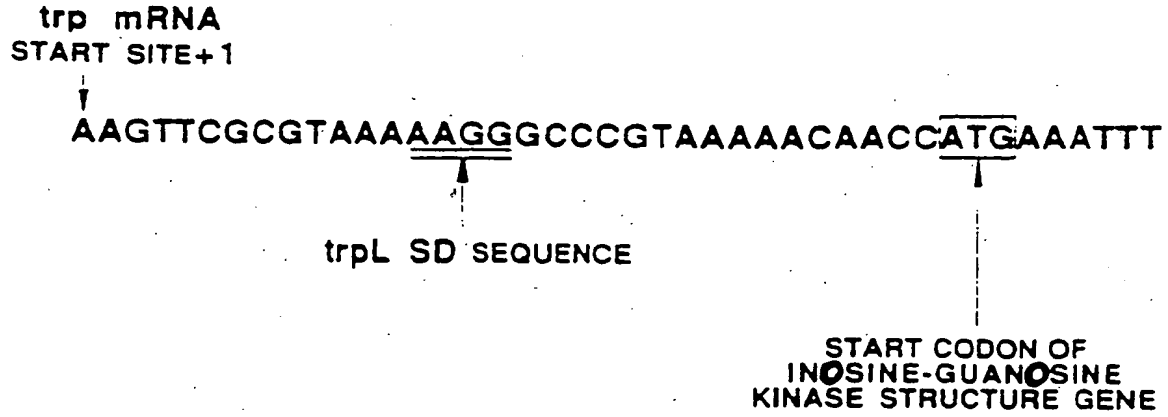
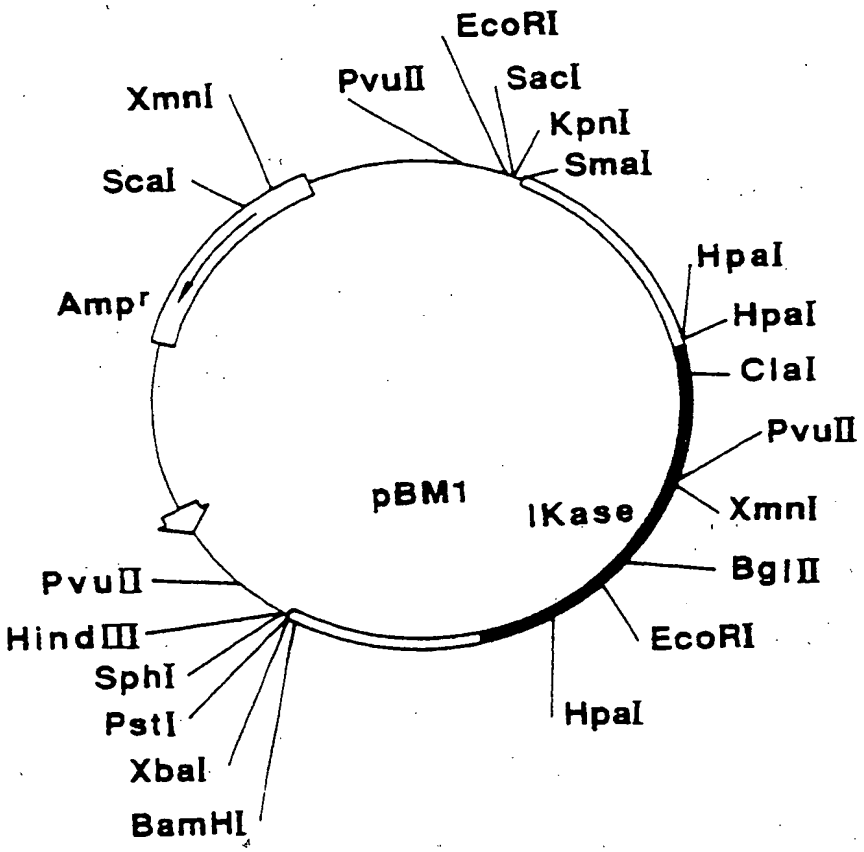

trp mRNA
START SITE+1
AAGTTCGCGTAAAAAGGGCCCGTAAAAACAACCATGAAATTT
trpL SD SEQUENCE
START CODON OF
INOSINE-GUANOSINE
KINASE STRUCTURE GENE

FIG. 9


EcoRI
PvuII
SacI
KpnI
SmaI
XmnI
ScaI
Ampr
HpaI
HpaI
ClaI
PvuII
XmnI
BglII
EcoRI
HpaI
pBM1
IKase
PvuII
HindIII
SphI
PstI
XbaI
BamHI